# RECENT PROGRESS IN OXYTOCIN RESEARCH

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*Sandoz Ltd., Basle, Switzerland*

# RECENT PROGRESS IN OXYTOCIN RESEARCH

By

**B. BERDE, M.D.**

*Pharmacological Laboratory*

*Sandoz Ltd*

*Basle, Switzerland*



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*Science is at no moment quite right,*
*but it is seldom quite wrong.*

Bertrand Russell

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B. Berde, M.D.

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# RECENT PROGRESS IN OXYTOCIN RESEARCH

# I

## INTRODUCTORY REMARKS

SINCE THE turn of the century, the biochemical approach has come to acquire an ever greater significance in the field of medicine. With the new techniques of biochemistry it has been possible to isolate and synthesise a large number of active substances occurring in the living organism. Moreover the processes of normal and pathological metabolism have been analysed and the mode of action of many biologically active substances has been elucidated. The information derived from these studies has made possible a planned search for new drugs. Progress has not, of course, been equally rapid in all fields. While many questions can be regarded as virtually settled, it is only in the last few years that research into the biologically active polypeptides has got properly under way, mainly because it has had to await the refined methods of modern chemistry. Such methods are gradually making these interesting compounds accessible to the physiologists and pharmacologists and, indeed, a few of them are already available to the clinicians. The progress that has been made in recent years in the field of posterior pituitary hormones, including oxytocin, bears witness to the great impetus that can be given to physiological and clinical research by a major biochemical advance.

Reviewing the most important stages in oxytocin research (Table I), we see that the effect of posterior pituitary gland extract on the uterus was discovered in 1906

(DALE), and its mammary gland effect shortly afterwards in 1910 (OTT and SCOTT) Nearly twenty years went by, however, before KAMM and his co-workers (1928) succeeded in obtaining a posterior pituitary extract, Pitocin, which consisted mainly of oxytocin, i.e., was largely, but not entirely free from vasopressin. Yet another score years passed before pure oxytocin was isolated from animal pituitary glands (LIVERMORE and DU VIGNEAUD, 1949). A

TABLE I

SOME IMPORTANT DATES IN OXYTOCIN RESEARCH

| | |
|---|---|
| 1906 | Action of posterior pituitary extracts on the uterus—H H Dale |
| 1910 | Action of posterior pituitary extracts on the mammary gland—I Ott and J C Scott |
| *1928:* | *A posterior pituitary extract (Pitocin) containing mainly oxytocin, i e, relatively free from vasopressin—O Kamm et al* |
| 1949 | Isolation of pure oxytocin—A H Livermore and V Du Vigneaud |
| 1953 | Synthesis of oxytocin—V Du Vigneaud *et al* |
| 1955 | Process for industrial synthesis of oxytocin (Syntocinon)—R Boissonnas *et al* |
| 1956 | Synthetic analogues of oxytocin—R Boissonnas *et al*, J Rudinger *et al* |

few years later its structural formula was elucidated (DU VIGNEAUD *et al*, 1953c, TUPPY, 1953) and, finally, oxytocin was synthesised (DU VIGNEAUD *et al.*, 1953b) DU VIGNEAUD and his colleagues thus won the distinction of having achieved the first synthesis of a polypeptide hormone—a fitting reward for years of patient research For this fine achievement, rightly hailed by the *British Medical Journal* as "a major triumph of the biochemists," DU VIGNEAUD was honoured with the Nobel prize for chemistry in 1955 In that same year a method was developed for the production of oxytocin on an industrial scale (BOISSONNAS *et al*, 1955), and since February 1, 1956, the synthetic hormone has been available to the clinician under the trade-name of Syntocinon. The first analogues of oxytocin were like

wise synthesised in 1956 (Boissonnas *et al.*, 1956; Rudinger *et al.*, 1956).

As the following chapters will show, the successful elucidation of the chemical aspects of the oxytocin problem was of special importance in furthering the progress that has been made in this field. It will be appropriate, therefore, to discuss first some questions relating to the chemistry of oxytocin. We shall then examine the findings that have been forthcoming from physiological and pharmacological studies and deal with some questions of clinical interest. The author has sought to trace a thread of coherence through the evolution of the oxytocin problem over the last few years, however, no attempt has been made to give an exhaustive review of the literature.

# II

## CHEMICAL STRUCTURE AND BIOLOGICAL ACTIVITY OF OXYTOCIN AND RELATED POLYPEPTIDES

OXYTOCIN is a polypeptide amide (DU VIGNEAUD *et al.*, 1953b) which, on hydrolysis, yields a mixture of eight amino-acids and ammonia. The components are arranged in peptide linkage to form a 20-membered ring of five amino-acids (a cyclic pentapeptide moiety containing cystine with one free amino group) and a side-chain of three amino-acids (Fig. 1). Oxytocin isolated from human, bovine and porcine posterior pituitary is chemically identical (LIGHT and DU VIGNEAUD, 1958). The molecular weight is 1007. An important chemical property of oxytocin is that the 20-membered ring can be reversibly opened by reduction of the -S.S- of the cystine residue to -SH HS-, i.e. to form two cysteine residues. The question naturally arises as to how far the ring structure contributes to the specific biological activity of oxytocin.* A pentapeptide amide which represents the cyclic moiety of oxytocin (Fig. 2) has been synthesised (RESSLER, 1956) and found to have a measurable, though only very weak

---

* The biological activity of oxytocin and related compounds is expressed in international units (I.U. = $10^3$ mU. = $10^6$ μU.). This unit is the specific oxytocic activity corresponding to that of 0.5 mg. of the International Standard Pituitary (Posterior Lobe) Powder.

Fig 1 The structural formula of oxytocin

biological effect 0 66%, 0 22% or 0% that of oxytocin, depending upon the method of bioassay employed Clearly, therefore, the side-chain of the oxytocin molecule is of considerable biological importance

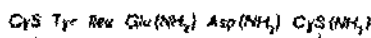



Fig 2 The sequence of the amino acids in the disulphide ring moiety of oxytocin

Vasopressin, the other posterior pituitary hormone (Fig. 3), is likewise an octapeptide amide (DU VIGNEAUD

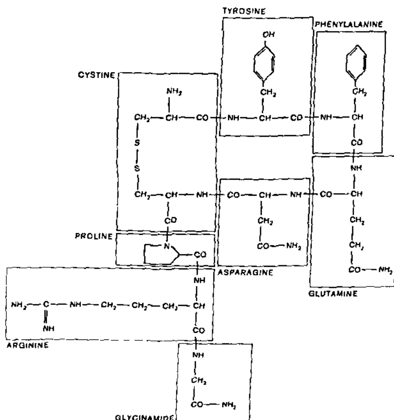



Fig 3 The structural formula of vasopressin This is the 'bovine' type of the hormone or 'arginine vasopressin" occurring in many animals and in man The "porcine" type of the hormone or "lysine vasopressin' found in the pig contains lysine instead of arginine in the side chain

*et al*, 1953a) with a 20-membered ring of five amino-acids and a side-chain of three amino acids Vasopressin differs from oxytocin only in two of its amino-acids instead of

*iso*-leucine in the ring structure it has phenylalanine, and instead of leucine in the side chain it has arginine (human, monkey, ox, camel, dog, sheep, rabbit, rat) or lysine (pig) (VAN DYKE *et al.*, 1957). As might be expected, therefore, pure vasopressin possesses a certain oxytocic activity (VAN

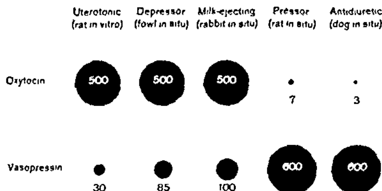



Fig 4 Diagram illustrating the biological activity of oxytocin and arginine vasopressin in different tests The surface areas are proportional to the potency in terms of the U S P standard per mg substance *The figures are those published by* VAN DYKE *et al* (1955)

DYKE *et al*, 1955) and pure oxytocin exerts very slight, but measurable vasopressor and antidiuretic effects (VAN DYKE *et al*, 1955, GYERMEK and FEKETE, 1955, BERDE and CERLETTI, 1956; KONZETT *et al.*, 1956). This can be seen from Figure 4 In addition to the uterotonic, i e. the true oxytocic effect (see Chapter V), two other properties are regarded as characteristic of oxytocin, namely its action on the mammary gland (see Chapter VI) and its depressor effect on avian blood pressure (see Chapter VII). The pressor and antidiuretic activities are typical properties of vasopressin.

Particular interest attaches to those synthetic oxytocin analogues which differ from the natural hormone in only one amino-acid (Fig 5), since a comparison of the pharmacological properties of such compounds gives some insight into the relationship between their chemical structure and biological activity.

| | |
|---|---|
| Synthetic oxytocin | CyS-Tyr-Ileu-Glu ($NH_2$)-Asp ($NH_2$)-CyS-Pro-Leu-Gly ($NH_2$) |
| Phenylalanyl analogue | CyS-Tyr-Phe Glu ($NH_2$)-Asp ($NH_2$)-CyS-Pro-Leu-Gly ($NH_2$) |
| Leucyl analogue | CyS-Tyr-Leu-Glu ($NH_2$)-Asp ($NH_2$)-CyS-Pro-Leu-Gly ($NH_2$) |
| Valyl analogue ( valyl oxytocin ) | CyS-Tyr-Val-Glu ($NH_2$)-Asp ($NH_2$)-CyS-Pro-Leu-Gly ($NH_2$) |
| Glutaminyl analogue | CyS-Tyr-Ileu-Glu ($NH_2$)-Glu ($NH_2$)-CyS-Pro-Leu Gly ($NH_2$) |
| Isoglutaminyl analogue | CyS-Tyr Ileu-Iglu ($NH_2$) Asp ($NH_2$) CyS Pro-Leu Gly ($NH_2$) |

(Iglu stands for isoglutamine by analogy to Ileu for isoleucine )

Fig 5 The sequence of amino acids in oxytocin and its analogues

Pharmacological studies of these oxytocin analogues have shown that quite minor changes in the oxytocin molecule can result in a loss of the specific biological activity, though this is by no means always the case Thus the polypeptide in which *iso*-glutamine replaces glutamine (RESSLER and DU VIGNEAUD, 1957) has none of the actions of oxytocin and vasopressin in the conventional tests. Similarly, the oxytocin analogue with glutamine replacing asparagine—one of a series synthesised in the Sandoz laboratories (BOISSONNAS *et al*, 1956)—is also practically without activity (BERDE *et al*, 1957) It can therefore be assumed that the glutaminyl and asparaginyl residues are of particular importance with regard to the specific biological activity of oxytocin

Not so the *iso* leucyl residue in the ring: if this is replaced by other amino-acid residues, the compounds obtained are biologically active, though their properties differ to a greater or lesser extent from those of oxytocin (BERDE *et al.*, 1957). For instance, the phenylalanyl analogue has a less powerful oxytocic effect and a greater vasopressor and antidiuretic activity than oxytocin. This is not surprising, for the phenylalanyl analogue is intermediate in chemical structure between oxytocin and vasopressin. it is an octapeptide comprising the cyclic moiety of vasopressin and the tripeptide amide side-chain of oxytocin Quantitative data on this analogue are given in Table II.

This same compound has also been prepared and studied in a number of biological tests by KATSOYANNIS (1957) who gave it the name "oxypressin" KATSOYANNIS (1957) likewise found this polypeptide to have a higher ratio of pressor to oxytocic activity than oxytocin; although, quantitatively, his findings differed somewhat from those published from this laboratory (BERDE *et al*, 1957).

The substitution of leucine for *iso*-leucine in the oxytocin molecule leads to essentially the same changes in activity as the replacement of *iso*-leucine by phenylalanine the oxytocin properties are attenuated and the vasopressin properties are enhanced On the whole, however, the leucyl analogue is less active than the phenylalanyl compound (Table II)

One of the compounds synthesised by BOISSONNAS *et al.* (1956) merits special attention, namely the oxytocin analogue with valine replacing *iso* leucine (Fig 6). This compound, "valyl oxytocin," has almost negligible pressor-antidiuretic activity, but retains high oxytocic potency; its activity in various tests can be seen in Table II The most

TABLE II

(Activity of 1 ml of Solutions of the Cyclic Octapeptides in Units of International Standard Pituitary (Posterior Lobe) Powder in Different Tests (Berde *et al*., 1957)

| | Isolated Rat Uterus | Chicken Blood Pressure | Milk-ejection Pressure (Rabbit Mammary Gland) | Cat Uterus *in situ* | Antidiuretic Activity (Non-anaesthetised Rats) | Pressor Activity (Spinal Cats) |
|---|---|---|---|---|---|---|
| Synthetic oxytocin | 8.0 (±0.22) | 8.1 (±0.16) | 8.3 (±0.23) | 10.7 (±0.9) | 0.09 (±0.05) | 0.07 |
| P-analogue | 2.7 (±0.11) | 2.2 (±0.08) | 5.8 (±0.37) | 2.4 (±0.3) | 2.8 (±1.2) | 0.5 |
| L-analogue | 0.25 (±0.01) | 0.33 (±0.01) | 2.2 (±0.44) | 2.3 (±0.4) | 0.15 (±0.08) | 0.15 |
| V-analogue ("valyl-oxytocin") | 2.8 (±0.11) | 3.2 (±0.08) | 15.0 (±0.75) | 16.7 (±1.3) | 0.04 (±0.02) | 0.015 |
| G-analogue | <0.0025 | <0.02 | 0.025 (±0.003) | 0 | 0 | 0 |
| | | | | (up to 1 ml /kg i.v.) | (up to 1 ml / 100g s.c.) | (up to 0.5 ml /kg. i.v.) |

P = phenylalanyl
L = leucyl
V = valyl
G = glutaminyl

interesting feature of valyl-oxytocin from a biological point of view is that it possesses a more powerful oxytocic activity *in vivo* than *in vitro*. Oxytocin preparations are

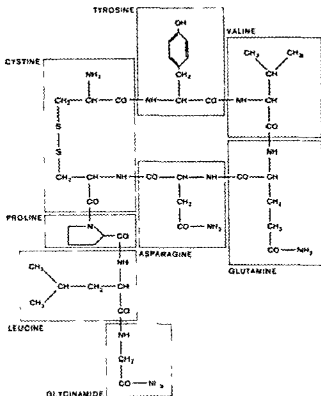



Fig. 6. The structural formula of valyl-oxytocin

usually standardised against International Standard Pituitary (Posterior Lobe) Powder, for which purpose both the *United States Pharmacopeia* (1955) and the *British Pharmacopoeia* (1953) recommend an assay method based on the depression of blood pressure in the chicken (Coon, 1939). An alternative test suggested in the *British Pharma-*

*copoeia* (1953) employs the rat uterus *in vitro* (HOLTON, 1948). If other methods are used to assay oxytocin—based for example on the cat uterus *in situ* or the milk-ejection pressure—practically the same values are obtained as with the pharmacopoeia methods. However, valyl-oxytocin behaves differently than the natural hormone: its oxytocic effect measured on the cat uterus *in situ* or on the milk-ejection pressure in the lactating rabbit is appreciably higher than the values found by the pharmacopoeia methods (BERDE *et al.*, 1957)



Fig 7 Characteristic posture of a doe rabbit during delivery the animal puts its head between its hind legs, licks its genitals before the expulsion of the foetus, then licks the new-born offspring and eats the placenta and the membranes

For this reason an attempt was made to devise a method which would make it possible to measure the potency of oxytocin-like substances under physiological con

ditions and on the same function on which such substances would act in man (BERDE and CERLETTI, 1958a). It is known that labour can be induced in pregnant rabbits near term by administration of a posterior pituitary extract (KNAUS, 1926), or of pure oxytocin (CSAPO, 1955).

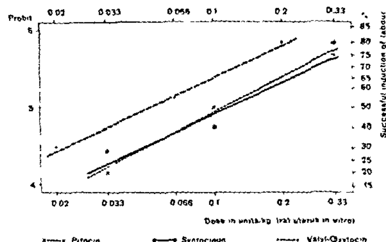



Fig 8 Dose response curves of Syntocinon (synthetic oxytocin) Pitocin (natural oxytocin) and valyl-oxytocin assayed by the induction of labour method In this test valyl-oxytocin is twice as potent as when assayed by the *conventional methods for the biological* standardisation of oxytocin (BERDE and CERLETTI 1958a)

Moreover, even in non-pregnant rabbits, oxytocin evokes a behaviour pattern which in many respects is reminiscent of labour (FAURE, 1957) A method was duly developed in which induction of labour in the rabbit is used to assay oxytocic substances the test substances are administered to rabbits on the 31st day of pregnancy and the time of onset of labour is recorded (Fig 7) Like other *in vivo* methods this new test gave a higher potency for valyl-oxytocin than the pharmacopoeia methods (Fig 8)

The phenomenon of higher activity *in vivo* is demonstrated to particular advantage when the uterotonic activity of valyl-oxytocin is compared in the same species both *in vivo* and *in vitro*. In the cat and in the rat the effect of valyl-oxytocin is some three times more powerful on the uterus *in situ* than on the isolated organ. In contrast, oxytocin has the same activity in both types of experiment. The fact that the two compounds should differ so radically in this respect is all the more interesting when we consider that valyl-oxytocin has only one methyl group less than oxytocin, but is otherwise identical in structure * The high *in vivo* oxytocic activity of valyl-oxytocin—which, incidentally, has recently been corroborated in the human female (Fig. 9) (SMYTH, 1958b)—is still more impressive when compared with the almost negligible pressor-antidiuretic effect of this analogue And, furthermore, the ratio of the oxytocic effect to the blood pressure lowering activity in the human female (see Chapter VII) is likewise higher (i e., clinically more favourable) for valyl-oxytocin than for oxytocin (SMYTH, 1958b). The implication of these findings is that the oxytocin molecule can be modified to give a substance which, from the obstetrician's viewpoint, is superior to the natural hormone

The valyl and leucyl analogues of oxytocin have also been synthesised by RUDINGER *et al.* (1956), but these

*Another instance has recently come to light of the way in which replacement of an amino acid residue by a valyl residue can markedly alter the properties of a protein In sickle cell anaemia, a serious blood disease, the haemoglobin in the erythrocytes differs in chemical structure from that of normal blood The difference involves only one of the nearly 300 ... cell haemo ... by a valyl

workers have not yet published detailed quantitative data on the biological activities of these compounds.

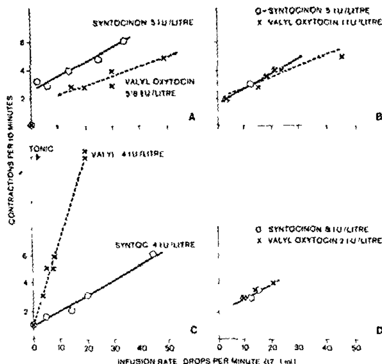



Fig 9 Comparison of synthetic oxytocin (Syntocinon) and valyl-oxytocin in four intra partum patients at term The activity of both preparations is given in International Units as determined by the pharmacopoeia methods In equal concentrations valyl oxytocin is considerably more potent than oxytocin (curve C) Diluted 8 times (curve A) valyl-oxytocin is noticeably less potent, and at 4 times dilution (curve D) there is no perceptible difference (SMYTH, 1958b)

Recapitulating pharmacological studies of a number of oxytocin analogues have revealed that if the glutaminyl or the asparaginyl residue in the oxytocin molecule is replaced by some other amino-acid all oxytocic potency is

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disulphide ring than oxytocin and vasopressin but none of their activity, inhibits the pressor effect of vasopressin on the rat blood pressure.

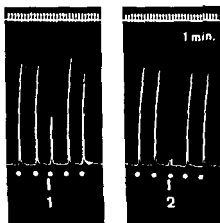



Fig. 11. Contractions elicited in the isolated rat uterus by oxytocin, inhibited by a nonapeptide. At intervals of 5 min (indicated by the white spots), 12 mU of synthetic oxytocin (Syntocinon) was introduced into the organ bath (50 ml) which was washed out when the contraction had taken place. At 1, 0.05 ml and at 2, 0.1 ml of nonapeptide solution was added to the bath, 30 seconds before the next dose of oxytocin. The smaller dose of nonapeptide reduced the amplitude of the contraction by about 50%, while the larger dose almost fully inhibited the contraction.

lost. On the other hand, replacement of the *iso*-leucine by other amino-acids yields octapeptides with modified biological activity. One of these octapeptides—valyl oxytocin—surpasses the natural hormone as an oxytocic.

*Research into synthetic higher homologues of oxytocin* has not yet progressed so far as the study of oxytocin analogues, but nevertheless one finding of some importance has emerged. A nonapeptide has recently been synthesised in the Sandoz laboratories which contains all eight amino acids of the natural hormone and, in addition, a second tyrosyl residue in the ring (Fig. 10). This nona

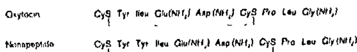



Fig. 10. The sequence of the amino acids in oxytocin and in a nonapeptide which inhibits some effects of oxytocin

peptide has been found to have a certain inhibitory effect on oxytocin when given shortly before the latter in some tests, e.g. *chicken blood pressure, rabbit and rat uterus in vitro* (Fig. 11) (GUTTMANN *et al.*, 1957; KONZETT, 1957). For the moment this is of purely theoretical interest. *What the clinician requires is an oxytocin inhibitor with a prolonged action in vivo which could be used for temporary suppression of uterine motility. None the less, these findings suggest that synthetic polypeptides related to oxytocin may aid us in our search for such a compound.*

*The nonapeptide may inhibit the action of oxytocin* by some competitive mechanism. It is tempting to speculate whether the inhibitory activity of this higher homologue is due to the larger size of the disulphide ring. This possibility has been hinted at by RESSLER and RACHELE (1958) who found that the *iso*-glutamine isomer of oxytocin ("*iso*-glutamine oxytocin"), which also has a larger

cause no secretion: extracts of the atrophied gland possess neither oxytocic nor pressor antidiuretic properties (Fisher, Ingram and Ranson, 1938). The most important connection is the hypothalamico-hypophyseal tract which is chiefly made up of fibres of the supraoptico-hypophyseal tract. These fibres

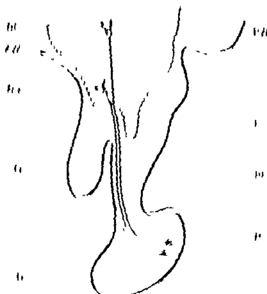


Fig. 14. Diagram of the neurosecretory pathways in the hypothalamus and the neurohypophysis in man. PV and SO neurosecretory cells representing the paraventricular nucleus and supraoptic nucleus: their nerve fibres run down the infundibular stalk (IS) into the infundibular process (P) of the neurohypophysis. AW and PW anterior and posterior walls of the hypothalamus. I infundibulum. O optic chiasma. G the glandular lobe of the hypophysis (adenohypophysis) ([illegible])

have their origin in the supraoptic and paraventricular nuclei and terminate in the neurohypophysis, principally in the posterior lobe (Fig. 14). In addition to the infundibular process (posterior lobe) the neurohypophysis

# III

## THE PRODUCTION OF OXYTOCIN IN THE ORGANISM

SINCE LOEWI'S (1921) classical investigations on the frog heart, it has been known that the endings of peripheral nerves release chemical mediators which transmit impulses from the nervous system to the organs The effect of acetylcholine liberated by cholinergic nerves is confined to the immediate vicinity of the nerve endings The mediators released by adrenergic nerves, on the other hand, are metabolised more slowly than acetylcholine and can therefore evoke systemic effects Noradrenaline, for example, is present in adrenergic nerves and is liberated at the endings of such nerves, a part of it finding its way into the blood stream and affecting the function of distant organs (for details, see VON EULER, 1956) Here, then, we have a hormone that is produced and liberated by peripheral nervous elements.

In recent years numerous investigations into the production of oxytocin and the production of posterior pituitary hormones in general, have pursued the possibility that such hormones might likewise be a product of nervous elements, namely of neurones in the hypothalamus The production of hormones in the central nervous system is called neurosecretion

It has been known for a long time that the hypothalamus and the pituitary gland are intimately connected If the posterior pituitary is denervated, it atrophies and

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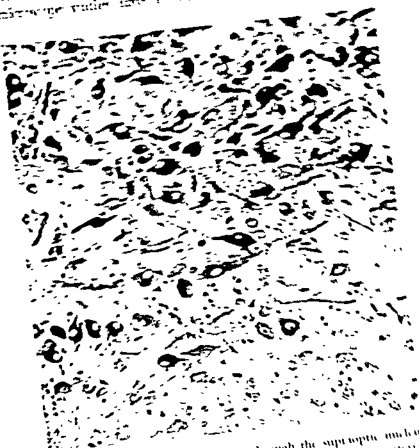


Fig. 13 Stained histological section through the supraoptic nucleus of the dog. The ganglion cells containing neurosecretory material appear dark (BARGMANN 1958)

ules are smaller than they appear under the light microscope. In the hypophysis of the rat (PALAY, 1955), mouse, rabbit (BARRY 1957), and cat (GREEN and VAN BREEMEN

includes the infundibular stem and the median eminence of the tuber cinereum (see Fig 33). There is no histological evidence to suggest that any of the cellular elements of the neurohypophysis are secretory cells (gland cells) capable of producing hormones. On the other hand, the nerve endings are intimately associated with the blood vessels (BODIAN, 1951). And, indeed, there is now much evidence to support the view that oxytocin and vasopressin are actually produced in the brain, or to be more precise in the anterior hypothalamus, and that they are merely stored in the neurohypophysis

Investigations by E. and B. SCHARRER (1928, 1932, 1954a, 1954b) and by BARGMANN (1951, 1954, 1958a) have shown that in both invertebrates and vertebrates, including mammals, certain hypothalamic neurones produce granules of neurosecretory material which are transported along the axons This neurosecretory material progresses along the axons at a rate of about 3 mm. per day. BARGMANN (1949) made the important discovery that the neurosecretory granules are selectively stained by GOMORI'S chrome-haematoxylin phloxine With this staining technique, the supraoptic and paraventricular nuclei of various animals, including mammals, can be shown to contain abundant neurosecretory material (Fig 13), and, moreover, the granules can be traced along the supraoptico-hypophyseal tract down to the perivascular nerve endings Indeed in one species, the giraffe, they have been observed in the capillaries (HANSTROM, 1957), but in other animals, e.g , the horse, no granules have been found in the lumen of the vessels (KIVALO and TALANTI, 1957) Be that as it may, the accumulation of neurosecretory material in the posterior pituitary, massed around the capillaries, has been demonstrated in all species investigated Incidentally, neurosecretory granules have even been demon

It was pointed out some years ago (e.g., by ABEL, 1924) that hypothalamic and posterior pituitary extracts possessed similar biological actions. When histological data began to accumulate, this question became the object of systematic research (HILD and ZETLER, 1951, 1952, 1953a, 1953b; VAN DYKE *et al.*, 1957). Extracts of the supraoptic and paraventricular nuclei and likewise of the tuber cinereum were found to possess oxytocic, antidiuretic and vasopressor properties, whereas the tissues surrounding these structures were devoid of hormone. The activity (i.e., hormone content) of these brain structures was, however, less than that of the neurohypophysis. This was first demonstrated in the dog, but it applies also to a number of other animals and to man (Tables III and IV). There is a certain correlation between the amount

TABLE IV

HORMONE CONTENT OF THE HUMAN POSTERIOR PITUITARY IN I.U. PER INDIVIDUAL (HILD AND ZETLER 1952)

| No | Vasopressin | Adiuretin | Oxytocin |
|---|---|---|---|
| 1 | 8.00 | 8.00 | 10.80 |
| 2 | 8.40 | 6.00 | 9.00 |
| 3 | 0.88 | 1.76 | 3.80 |
| 4 | 32.00 | 28.00 | 30.80 |
| 5 | 14.40 | 10.28 | 12.00 |
| 7 | 18.72 | 15.00 | 9.60 |
| 8 | 5.20 | 6.00 | 4.80 |
| 10 | 4.32 | 6.40 | 3.84 |
| Mean | 11.49 | 10.18 | 10.33 |
| $\pm\sigma$ | 9.40 (82%) | 7.13 (75%) | 8.44 (82%) |
| $\pm\varepsilon$ | 3.55 (31%) | 2.88 (29%) | 3.19 (31%) |

$\sigma$ = standard deviation
$\varepsilon$ = standard error of the mean

1955), the granules range in diameter from 50 to 200 mμ; in ox hypophysis they show a greater variation in diameter (SCHIEBLER, 1952). Recent data published by BARGMANN (1958b) indicate a diameter of 120 to 180 mμ for the granules in the cat.

It is reasonable to suppose on the strength of these histological studies alone, that the so called posterior pituitary hormones might quite well be hypothalamic hormones, generated in the supraoptic and paraventricular nuclei, transported along the axons, and released into the blood vessels of the neurohypophysis Additional evidence to support this view is forthcoming from physiological investigations

TABLE III

HORMONE CONTENT OF THE ANTERIOR HUMAN HYPOTHALAMUS IN I U PER INDIVIDUAL (HILD AND ZETLER, 1952)

| No | Vasopressin | Adiuretin | Oxytocin |
|---|---|---|---|
| 1 | 0 129 | 0 166 | 0 480 |
| 2 | 0 316 | 0 122 | 0 257 |
| 3 | 0 120 | 0 018 | 0 251 |
| 4 | 0 240 | 0 274 | 0 324 |
| 5 | 0 210 | 0 120 | 0 164 |
| 6 | 0 160 | 0 008 | 0 034 |
| 7 | 0 208 | 0 167 | 0 120 |
| 8 | 0 520 | 0 184 | 0 600 |
| 9 | 0 128 | 0 101 | 0 120 |
| 10 | 0 183 | 0 105 | 0 180 |
| Mean | 0 228 | 0 135 | 0 255 |
| $\pm\sigma$ | 0 1095 (48%) | 0 0672 (50%) | 0 1810 (71%) |
| $\pm\epsilon$ | 0 0346 (15%) | 0 0238 (18%) | 0 0550 (22%) |

$\sigma$ = standard deviation

$\epsilon$ = standard error of the mean

solubility studies that the neurosecretory material cannot be identical with the hormones, but must be regarded as a carrier substance (HILD and ZETLER, 1953b). It is not yet clear whether the GOMORI-positive carrier substance is identical with "neurophysine", a biologically inert protein present in some neurohypophyseal extracts, which is said to be somehow associated with oxytocin and vasopressin (ACHER, 1958).

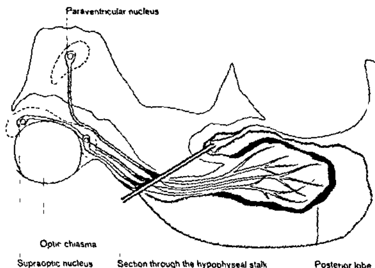



Fig 14 Diagram showing what occurs when the hypophyseal stalk is cut In the proximal stumps of the fibres descending from the supraoptic nucleus and the paraventricular nucleus there is an accumulation of neurosecretory material The distal ends of the fibres are devoid of granulation (BARGMANN, 1958)

Extracts of the supraoptic nucleus, like extracts of the paraventricular nucleus, possess both oxytocic and pressor-antidiuretic properties (VAN DYKE *et al.*, 1957) This would seem to indicate that oxytocin and vasopressin are not produced in different, clearly defined regions of the

of neurosecretory material that can be demonstrated and the biological activity of the extracts (HILD and ZETLER, 1952), which implies that there is some connection between hormone production and the generation of neurosecretory material. In keeping with this, it has recently been *observed that chlorpromazine in doses affecting* diuresis, leads to a reduction in the amount of neurosecretory material in the neurohypophysis (KIVALO *et al.*, 1958)

A cogent piece of evidence in support of the neurosecretory theory is available in studies on dogs whose pituitary stalks had been sectioned (HILD and ZETLER, 1953a) Mechanical interruption of the hypothalamo-hypophyseal tract leads to a thickening of the fibres in the proximal part of the pathway and an accumulation of neurosecretory material in the thickened stumps of the fibres. In the distal part of the pathway the amount of GOMORI-positive material decreases This would suggest *that there is a flow of neurosecretory material along the* fibres (Fig 14) The hormone content of the hypothalamus is markedly reduced after a lengthy period of water deprivation When such thirsting stalk-sectioned animals are supplied with water, the amount of histologically demonstrable neurosecretory material increases proximal to the point of sectioning, and the biological activity of hypothalamic extracts rises Distal to the point where the stalk was sectioned, there is no increase in the quantity of neurosecretory material, nor is there a rise in the biological activity of extracts (HILD and ZETLER, 1953a). It might therefore be imagined that the granules revealed by the specific staining technique were the actual hormones, i e, *that the neurosecretory material was perhaps* identical with the proteohormones shown up by the GOMORI stain. However, it has been demonstrated by

solubility studies that the neurosecretory material cannot be identical with the hormones, but must be regarded as a carrier substance (HILD and ZETLER, 1953b). It is not yet clear whether the GOMORI-positive carrier substance is identical with "neurophysine", a biologically inert protein present in some neurohypophyseal extracts, which is said to be somehow associated with oxytocin and vasopressin (ACHER, 1958).

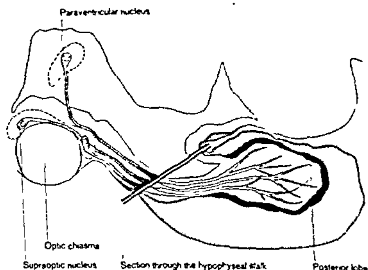



Fig 11 Diagram showing what occurs when the hypophyseal stalk is cut In the proximal stumps of the fibres descending from the supraoptic nucleus and the paraventricular nucleus there is an accumulation of neurosecretory material. The distal ends of the fibres are devoid of granulation (BARGMANN, 1958)

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hypothalamus. Nevertheless certain findings (OLIVECRONA, 1957) suggest that the paraventricular nucleus is of particular importance as regards the production of oxytocin, at any rate in the rat: if this nucleus is destroyed, the oxytocin content of the pituitary gland decreases, whereas the vasopressin content is unchanged. The blood of such animals likewise contains less oxytocin than that of normal animals (DUGGAN and REED, 1958).

Centrifuge studies (PARDOE and WEATHERALL, 1955) have revealed that the hormones in the pituitary gland are probably bound to mitochondria and not to microsomes; moreover oxytocin and vasopressin seem to be contained in different mitochondria. This has an important bearing on the question of whether the hypothalamus produces two different hormones with a molecular weight of about 1,000 or whether the protein with a molecular weight of about 30,000 extracted by VAN DYKE *et al* (1942) is the true pre-formed hormone (1 oxytocic unit per 61 μg) This protein possesses all the biological properties of the posterior pituitary hormones, and if it were the true hormone, this would mean that the oxytocin (mol wt. 1,007, 1 oxytocic unit per 2 μg) and vasopressin isolated by DU VIGNEAUD were merely fragments of the large molecule

However, VAN DYKE's protein can be resolved into two fractions, one with oxytocic, the other with vasopressor-antidiuretic properties, by gentle methods such as electrophoresis and counter-current distribution which do not involve hydrolysis (ACHER *et al*, 1956a, ACHER and FROMAGEOT, 1957)

There is additional evidence to suggest that the posterior pituitary hormones are discrete entities in some animal species the ratio of vasopressor to oxytocic activity varies greatly from one part of the neurosecretory system

to another; and, moreover, the vasopressin:oxytocin ratio in a given part of the neurosecretory system varies from one species to the next, sometimes quite considerably (DICKER and TYLER, 1953b; VAN DYKE *et al.*, 1957). Thus in the dog the ratio of vasopressin to oxytocin is 15 in the paraventricular nucleus, 24 in the supraoptic nucleus, 14 in the tuber cinereum, and 1.5 in the posterior lobe of the pituitary. This ratio in the hypothalamus is 17 for the dog, 5 for the rat, and 1.1 for the camel. In the rat the vasopressin:oxytocin ratio shows characteristic changes during the oestrous cycle (Fig. 15) (HELLER, 1957a), parturition, and lactation (DICKER and TYLER, 1953a; ACHER *et al.*, 1956b). The variable ratio of vasopressor to oxytocic activity of human blood (BISSET and LEE, 1957) is additional evidence that the octapeptides are the actual hormones and that they are produced largely independently of each other.

Many stimuli are known to bring about a release of oxytocin: dilatation of the cervix and body of the uterus, coitus or mechanical stimulation of the uterus and vagina, suckling or mechanical stimulation of the mamilla, certain emotional stimuli, thirst or a rise in the osmotic concentration of the blood, acetylcholine, nicotine, anaesthetics; electrical stimulation of the supraoptico-hypophyseal tract. Apparently each stimulus that causes secretion of oxytocin elicits a simultaneous release of vasopressin. This is true of coitus (HARRIS and PICKLES, 1953; FRIEBERG, 1953), suckling (CROSS, 1951; PEETERS and COUSSENS, 1950; KAILIALA and KARVONEN, 1951) and milking (COWIE and FOLLEY, 1957), thirst (SIMON, 1934) and osmotic stimuli (ABRAHAMS and PICKFORD, 1954; HOLLAND *et al.*, 1958), emotional factors (Fig. 16), acetylcholine (ABRAHAMS and PICKFORD, 1954), nicotine (BISSET and WALKER, 1957), anaesthetics (CHAUDHURY and WALKER, 1958) and stimu

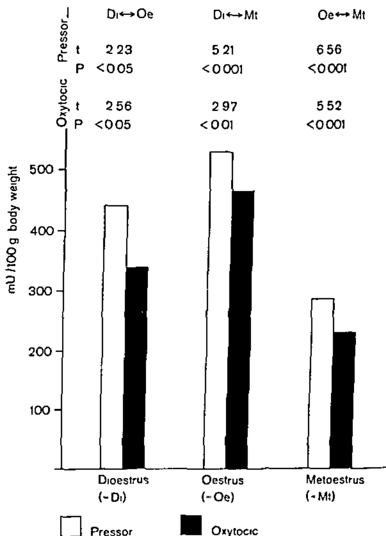



Fig 15 Pressor and oxytocic activity in the pituitary glands of adult female rats at different stages of the oestrous cycle (Heller, 1957a)

lation of the hypothalamus (HARRIS, 1948, 1955, ANDERSSON, 1957)

The mechanism of action of most of these stimuli is not yet understood. It is, however, known that the supraoptic and paraventricular nuclei are situated in that part of the hypothalamus which is sensitive to local osmotic stimuli (VERNEY, 1958) The injection of eserine or diisopropyl fluorophosphate into the supraoptic nucleus of the

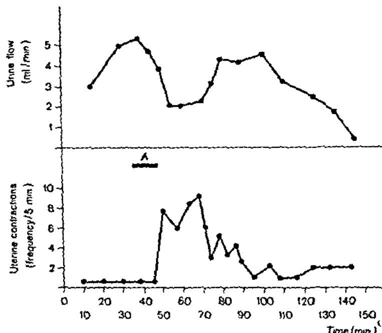



Fig 16 The effect of emotion on urine flow and uterine motility in a dog *Upper record urine flow Lower record frequency of* uterine contraction At zero time 300 ml water by mouth During period A preparations were made for an intracarotid injection which was not given Following this emotional stimulus decrease of urine *secretion (release of vasopressin)* as well as *increase of* uterine motility (release of oxytocin) can be observed (ABRAHAMS and PICKFORD, 1954)

dog causes increased uterine motility, from which it can *be assumed that cholinergic transmission is involved in* the central mechanisms responsible for the liberation of oxytocin (ABRAHAMS and PICKFORD, 1956). Studies with nicotine and hexamethonium have shown, however, that this particular synaptic transmission mechanism differs from that in the peripheral autonomic ganglia (BISSET and WALKER, 1957; WALKER, 1958)

Irrespective of the "logical purpose" of a given stimulus, the amount of oxytocin released from the neurohypophysis is always greater than the amount of vasopressin The ratio varies from 4 . 1 for electrical stimulation of the hypothalamus to 100 . 1 for suckling (HARRIS, 1955). For the time being this phenomenon remains an enigma If oxytocin and vasopressin were always released in constant proportions, it could be assumed that the cytochemical processes by which they are produced were so intimately geared that the two hormones could only *be built up and released in a fixed ratio* However, this is not the case: the organism appears to be able to regulate the production and liberation of each hormone separately Why then is oxytocin released along with vasopressin after an osmotic stimulus and why are both oxytocin and vasopressin released during suckling? Could it be that some sort of interaction between the two polypeptides at the effector organs is of greater importance than we suspect?

# IV

## THE FATE OF OXYTOCIN

Oxytocin released from the neurohypophysis is carried in the blood stream to the organs where it exercises its specific biological actions It is still not known with certainty in what form oxytocin is transported in the blood, but there are grounds for believing that it enters into loose combination with the serum proteins: in dialysis experiments (Heller, 1957a) oxytocin disappears at a faster rate from Krebs-Eggleston solution than from human plasma (Fig 17) Inulin which is not bound to the serum proteins diffuses out of both systems at the same rate.

The estimation of oxytocin in biological fluids acquires a special importance when we come to study the fate of the hormone. Quantitative data on the oxytocin content of the blood show considerable variation and are often difficult to interpret. This is due, in part at least, to difficulties inherent in the assay procedure Present-day methods for determining the oxytocin content of the blood (Bisset and Walker, 1954, Bisset *et al*, 1956) are based on measurement of that part of the uterotonic activity of blood extracts which is not due to other substances known to act on smooth muscle, e g potassium ions, histamine, 5-hydroxy-tryptamine, acetylcholine or adrenaline, and is abolished by thioglycolate This method is founded on sound biological principles, nevertheless it lacks the high degree of certainty that could be attained by means

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son, 1956) and from the hypothalamus—but not the pituitary gland—of various animals (ROBERTSON and HAWKER, 1957). Unlike oxytocin this substance is not inactivated by thioglycolate. Clearly, this finding still further complicates the interpretation of data on the oxytocic activity of blood and tissue extracts.

In the rat blood from the pituitary gland drains into the external jugular vein, so that the blood in this vessel would be expected to have a higher oxytocin concentration than that in other veins and arteries In anaesthetised rats the oxytocin concentration of blood from the external jugular vein ranged from 2 to 7 mU /ml (BISSET and WALKER, 1954) In the same vein in the cow, the oxytocin concentration was 120 to 300 μU./ml. and increased after stimulation of the uterus and vagina to as high as 420 to 850 μU /ml (FITZPATRICK, 1957). In man blood from the pituitary gland finds its way into the internal jugular vein The oxytocin concentration of blood from this vein was about 1 2 mU /ml in anaesthetised men and women (BISSET *et al*, 1956) In non-anaesthetised subjects the oxytocin content of blood from this vein was lower (Table V), while that of blood from peripheral veins was still less always below 0 4 mU /ml (BISSET and LEE, 1957) Other investigators (HAWKER and ROBERTSON, 1956) have found considerably higher oxytocin concentrations in female venous blood· 0 2 to 7 8 mU /ml in non-pregnant women, 0 95 to 43 mU /ml in pregnant women, and 0 1 to 1.17 mU /ml during the first stage of labour If we compare the values of BISSET and LEE (1957) for non pregnant women with those of HAWKER and ROBERTSON (1956), we see that the discrepancy is of the order of 1 . 10

Oxytocin is eliminated by inactivation or excretion. If strong uterine contractions are induced in a woman

of a specific chemical method of detecting oxytocin in the blood, if any such method were available. The shortcomings of the biological method are evident from the fact that if a known amount of oxytocin is added to the blood in vitro and an oxytocin assay is carried out, the value found is invariably too low. This can be seen from the last column of Table V Moreover, it has recently been reported that a second oxytocic substance can be extracted from human female blood (HAWKER and ROBERT-

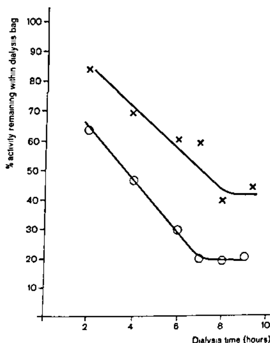



Fig 17 Dialysis to equilibrium of synthetic oxytocin (Syntocinon) x——x in human plasma and o——o in Krebs Eggleston phosphate saline buffer Initial concentration of oxytocin in dialysis bag 100 mU /ml Nojax casing (8/32) was used as the dialysis membrane It was rotated at 200 r p m at room temperature Activity was estimated by means of (4 + 4) assays on the rat uterus (HELLER, 1957a)

son, 1956) and from the hypothalamus—but not the pituitary gland—of various animals (Robertson and Hawker, 1957) Unlike oxytocin this substance is not inactivated by thioglycolate. Clearly, this finding still further complicates the interpretation of data on the oxytocic activity of blood and tissue extracts.

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Oxytocin is eliminated by inactivation or excretion If strong uterine contractions are induced in a woman

TABLE V

ENDOGENOUS OXYTOCIC AND ANTIDIURETIC ACTIVITY IN CENTRAL AND PERIPHERAL BLOOD FROM CONSCIOUS PATIENTS AND RECOVERY OF ADDED HORMONE (BISSET AND LEE, 1957)

| Case | Sex | Age (yr) | Condition | Endogenous activity in the blood | | | | | | Recovery of added hormone |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Central | | | Peripheral | | | |
| | | | | Source | O | A | Source | O | A | |
| 1 | F | 56 | Cirrhosis | I J V | 1 2 | <0 003 | F A | 0 2 (0 6) | <0 003 | 42% |
| 2 | M | 45 | Infective hepatitis | I J B | 0 3 | <0 007 | M C V | 0 1 (0 3) | <0 008 | 78% |
| 3 | M | 56 | Cirrhosis (+ amyloidosis) | I J B | 0 0 (0 6) | <0 003 | R A | 0 0 (0 2) | <0 004 | N E |
| 4 | M | 40 | Cirrhosis with ascites | I J B | 0 1 | 0 023 | M C V | <0 3 | <0 013 | 43% |
| 5 | F | 52 | Cirrhosis | S V C | <0 4 | <0 014 | M C V | <0 5 | <0 015 | 44% |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 6 | F | 45 | Cirrhosis with ankle oedema | I J A | 0.6 (1.1) | 0.019 | M C V | 0.4 (1.4) | <0.003 | 83% |
| | | Mean | | | <0.4 | <0.012 | | <0.3 | <0.008 | 58% |

O = oxytocic activity in terms of oxytocin (mU per ml )
A = antidiuretic activity in terms of vasopressin (mU per ml )
I J A , I J B = internal jugular vein or bulb
M C V = median cubital vein

S V C = superior vena cava.
F A = femoral artery
R A = right atrium
N E = not estimated

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ENDOGENOUS OXYTOCIC AND ANTIDIURETIC ACTIVITY IN CENTRAL AND PERIPHERAL BLOOD FROM CONSCIOUS PATIENTS AND RECOVERY OF ADDED HORMONE (BISSET AND LEE, 1957)

| Case | Sex | Age (yr) | Condition | Endogenous activity in the blood | | | | | | Recovery of added hormone |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Central | | | Peripheral | | | |
| | | | | Source | O | A | Source | O | A | |
| 1 | F | 56 | Cirrhosis | I J V | 1.2 | <0.003 | F A | 0.2 (0.6) | <0.003 | 42% |
| 2 | M | 45 | Infective hepatitis | I J B | 0.3 | <0.007 | M C V | 0.1 (0.3) | <0.008 | 78% |
| 3 | M | 56 | Cirrhosis (? amyloidosis) | I J B | 0.0 (0.6) | <0.003 | R A | 0.0 (0.2) | <0.004 | N.E. |
| 4 | M | 40 | Cirrhosis with ascites | I J B | 0.1 | 0.023 | M C V | <0.3 | <0.013 | 43% |
| 5 | F | 52 | Cirrhosis | S V C | <0.4 | <0.014 | M C V. | <0.5 | <0.015 | 44% |

body is dependent upon the kidneys and organs of the splanchnic vascular area; in lactating rats the oxytocin clearance is not solely dependent on this group of viscera. At all events, cell-free extracts of kidney, liver and intestine destroy the posterior pituitary hormones (HELLER, 1957b).

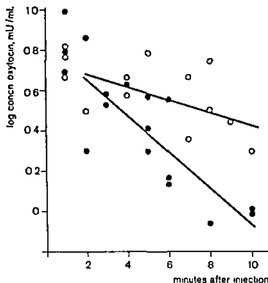



Fig. 18 The disappearance of injected oxytocin from the blood of rabbits. The animals were anaesthetised with urethane and blood was collected from the carotid artery. 200 mU/100 g oxytocin were injected into the ear vein. • normal rabbits, o rabbits whose kidney vessels had been ligated. (CHAUDHURY and WALKER, 1957)

A factor inactivating oxytocin can likewise be demonstrated in human placenta (HAWKER, 1956). Extracts prepared from fresh placenta with physiological saline will inactivate considerable amounts of oxytocin when incubated with the hormone at 37°C. The amount of hormone inactivated depends upon the concentration of the

near to term by intravenous drip infusion of oxytocin and the infusion is interrupted, the activity of the uterus falls by 50 per cent within about 15 minutes (CALDEYRO-BARCIA *et al.*, 1957b). This gives a good indication of the duration of action of oxytocin on the human uterus, but it is hardly permissible to draw any very definite conclusion concerning the elimination of oxytocin from observations of this kind. The persistence of an effect does not necessarily mean that the substance which elicited the effect is still present in an intact form in the organ affected Indeed another investigator (MULLER, 1958b) using a different—but also indirect—technique came to the conclusion that in the pregnant woman near to term a small dose of oxytocin has a half-life of about 1 minute. Both findings suggest that oxytocin is rapidly metabolised

A similar inference can be drawn from experiments by CHAUDHURY and WALKER (1957) who administered a known amount of oxytocin to rabbits and followed its rate of disappearance from the blood 10 minutes after intravenous administration of 200 mU oxytocin per 100 g body weight, the hormone could no longer be detected in the blood The half-life was 3 3 minutes In this respect oxytocin resembles vasopressin which likewise disappears rapidly from the blood If the renal blood vessels are ligated, the half life of oxytocin increases to 9 7 minutes (Fig 18) Though it is known that part of the injected oxytocin is excreted in the urine (LARSON, 1935), most of it would appear to be inactivated in the kidney tissues, as is the case with parenterally administered vasopressin which is inactivated by the kidneys and by the organs of the splanchnic vascular area (GINSBURG and HELLER, 1953) *Recently published data by* GINSBURG and SMITH (1958) suggest that in male rats, as well as in female rats in oestrus, the clearance of oxytocin from the

aroused a great deal of interest Further work has been carried out in this field (Werle *et al.*, 1941, 1950, 1951, 1956, Semm, 1953, 1955, 1958, Page, 1946, 1947) on the assumption that the inactivation is due to an enzyme ("oxytocinase") This property of pregnant serum to in-

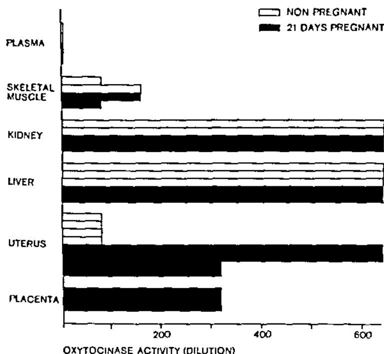



Fig 20 Comparison of oxytocinase activity in pregnant and non pregnant rat tissue The activity of uterine tissue is increased in pregnancy (Sawyer, 1954)

activate oxytocin can only be demonstrated in man and monkeys Attempts to detect any such property in the serum of a large number of other species (the rat, guinea *pig, rabbit, dog, pig, cow and horse) during pregnancy* have failed The inactivating factor in the serum appears

extract (Fig. 19). All placental extracts examined in this laboratory showed this activity which was of the same order for a number of specimens. The action is not strictly specific for oxytocin, since the extracts will also

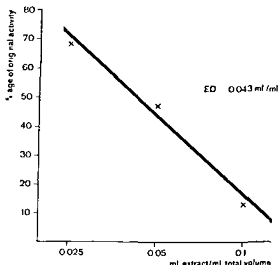



Fig 19 Inactivation of synthetic oxytocin (Syntocinon) by a physiological saline extract of human placenta A 0 1 I U /ml solution of Syntocinon was incubated with various amounts of the extract at 37°C for 60 minutes, whereupon the residual activity was determined 1 ml of extract corresponded to 0 8 g of placental tissue, dry substance = 3%, total-N = 4 08 mg /ml, protein-N = 3 86 mg /ml

inactivate valyl-oxytocin (BERDE and ZEHNDER, 1958). It is interesting to note that the capacity of the rat uterus to inactivate oxytocin shows a certain increase during pregnancy (SAWYER, 1954) The same does not apply to skeletal muscle, liver and renal tissue (Fig 20)

The discovery that the serum of pregnant women is able to inactivate oxytocin and that its capacity to do so increases as pregnancy advances (FEKETE, 1930, 1932),

findings in mind, the changes in oxytocinase activity could be regarded as one of nature's protective measures for keeping the uterus at rest during pregnancy. The oxytocinase would guard the uterus against oxytocin present in the blood and released from the neurohypophysis in considerable amounts in response to certain stimuli. It has actually been demonstrated that when oxytocin is infused for five to ten hours, the oxytocinase activity of the plasma increases (CALDEYRO-BARCIA and POSEIRO, 1958).

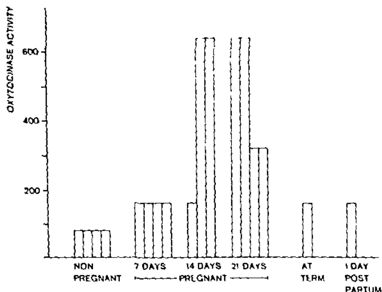



Fig. 21 Oxytocinase activity of the myometrium in rats at different stages of pregnancy (SAWYER, 1954)

This enzymatic mechanism would be an extra-uterine protective arrangement during pregnancy. It would be complementary to the well-known hormonal mechanism protecting the uterus (KNAUS, 1930), whereby uterine irritability is reduced, possibly as a result of suboptimal intra-

to differ from the inactivating factors occurring in various other parts of the body, e.g. in the erythrocytes, pancreas and ovaries. The serum factor does not pass from the maternal into the foetal circulation. When pregnant serum is subjected to vertical electrophoresis, oxytocinase is found on the cathode side of the albumin stream. The large molecules of serum oxytocinase cannot be dialysed, the enzyme is precipitated by 33 per cent saturation with ammonium sulphate, is unaffected by ultra-violet radiation, but is sensitive to heat. It is rapidly destroyed by boiling and is slowly inactivated by heating at 57°C. Oxidation also leads to loss of activity. The oxytocin inactivation curve conforms to that of a first order reaction Above 10°C the rate of the reaction increases with rising temperature The optimum pH lies between 6 5 and 7.5 and is rather sharply defined Cysteine and glutathione activate serum oxytocinase, but chelating agents, such as ethylenediamine tetraacetate, inhibit the enzymatic action Managanese, cobalt and zinc ions restore the lost activity Another chelating agent, 8-hydroxyquinoline, likewise inhibits serum oxytocinase Cyanide, however, does not affect the enzymatic reaction

For the time being it is difficult to say anything about the physiological significance of the oxytocin-inactivating factor of pregnant serum This is mainly because the different authors disagree as to the serum oxytocinase activity in the last weeks of pregnancy and during labour

According to DICKER and TYLER (1956) the capacity of pregnant serum to inactivate oxytocin decreases from the 28th week, and in most cases no such activity can be detected at term. There is a certain parallel between this finding and the observation that the capacity of the rat uterus to inactivate oxytocin increases during gestation, but is low at term (Fig. 21) (SAWYER, 1954). With these

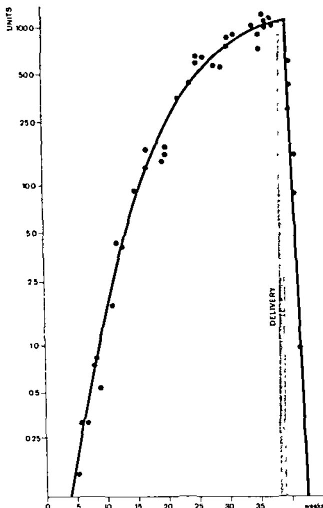



Fig 22 Plasma oxytocinase levels in normal pregnancy Ordinate units per ml plasma (values below 0 1 not illustrated) Abscissa weeks of pregnancy, dating from conception (PAGE, 1946).

cellular potassium ion concentration and the effect of the latter on the cell membrane (CSAPO, 1956). The decline in serum oxytocinase activity near to term would provide a convenient explanation of the increase in uterine sensitivity to oxytocin.

It must, however, be pointed out that most investigators have not found a decrease in serum oxytocinase activity at the end of pregnancy, but the opposite. According to WERLE and SEMM (1956), oxytocinase activity can be detected with certainty in the serum from the second month of pregnancy. It remains at roughly the same level from the third to the eighth month and then begins to increase again until at birth serum oxytocinase activity is sixty times higher than at the end of the second month According to another investigator (PAGE, 1946), oxytocinase activity is a thousand times higher in the thirty-eighth week than in the fourth week. He found a continuous rise in serum oxytocinase activity (Fig 22) without a plateau between the third and eighth month Yet another team of investigators (CALDEYRO-BARCIA and POSEIRO, 1958) has found oxytocinase activity to be at a maximum at term Hence the increasing sensitivity of the uterus to oxytocin towards the end of pregnancy can hardly be explained on the basis of changes in serum oxytocinase activity According to most investigators the inactivating factor only begins to decline after delivery

TUPPY and NESVADBA (1957) have recently shown that the effect of pregnant serum on the oxytocin molecule is probably to open the ring between the cystine and the tyrosine residue (Fig 23) From this it would appear that the inactivating factor is an aminopeptidase And, in fact, aminopeptidase substrates, viz. the β-naphthylamides of glycine, DL-alanine, L-leucine and L-cystine, were hydrolysed more rapidly by the serum of pregnant women in

TABLE VI

HYDROLYSIS OF AMINO-ACID β-NAPHTHYLAMIDES BY THE SERA OF PREGNANT AND NON-PREGNANT WOMEN. THE ENZYMATIC ACTIVITY OF THE SERA WAS DETERMINED AT pH 7.4 AND IS GIVEN IN MG. β-NAPHTHYLAMINE/100 ML. SERUM/HOUR (TUPPY AND NESVADBA, 1957)

| Sera | | Substrate β-Naphthylamide of | | | | | |
|---|---|---|---|---|---|---|---|
| | | Glycine | DL-Alanine | L-Leucine | L-Cystine | L-Cysteine | DL-Methionine |
| 37 to 40th week pregnant women | S 43 | 16.25 | 79.2 | 47.2 | 6.05 | 0.60 | 2.75 |
| | S 44 | 19.03 | 81.2 | 49.6 | 5.80 | 0.70 | 3.20 |
| | S 45 | 16.40 | 77.6 | 50.4 | 5.05 | 0.58 | 2.95 |
| Mean ($M_1$) | | 17.23 | 79.3 | 49.1 | 5.63 | 0.63 | 2.97 |
| Non-pregnant women | S 71 | 13.7 | 44.8 | 15.2 | 0.50 | 0.58 | 3.6 |
| | S 72 | 7.35 | 26.1 | 12.8 | 0.40 | 0.56 | 2.5 |
| | S 73 | 5.85 | 25.6 | 13.6 | 0.40 | 0.40 | 2.25 |
| Mean ($M_2$) | | 8.97 | 32.2 | 13.9 | 0.43 | 0.51 | 2.8 |
| $M_1/M_2$ | | 1.9 | 2.5 | 3.5 | 13.1 | 1.2 | 1.1 |

the thirty-seventh to fortieth week than by the serum of non-pregnant women (Table VI). Since the activity of the serum varied depending upon the enzyme substrate employed, it must be assumed that the serum contained more than one aminopeptidase. This type of investigation takes us an important step towards a solution of the "oxytocinase" problem, for it makes use of well-defined reactions permitting accurate chemical determination of the various aminopeptidases in the serum at different stages of pregnancy.

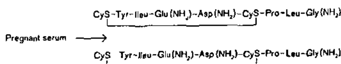



Fig 23 The action of dialysed pregnant serum on the oxytocin molecule (TUPPY and NESVADBA, 1957) The inactivating principle acts on the peptide link between the cystine residue and the tyrosine residue, opening the ring

It is still not certain, however, that the aminopeptidases investigated by TUPPY and NESVADBA (1957) are identical with the so-called serum oxytocinase of other workers In the first place, the serum of non-pregnant women has been found to possess a quite appreciable aminopeptidase activity, whereas earlier investigators were unable to detect any serum oxytocinase in non-pregnant women Furthermore, the most active enzyme, that hydrolysing *L-cystine di-β-naphthylamide*, shows only thirteen times greater activity (at pH 7 4) in pregnant women near to term than in non-pregnant women, whereas serum oxytocinase activity has been reported to be sixty, indeed as much as a thousand times higher during pregnancy. Then again, the aminopeptidases studied by TUPPY are inhibited by cyanide and manganese ions which are with-

TABLE VI

HYDROLYSIS OF AMINO-ACID $\beta$-NAPHTHYLAMIDES BY THE SERA OF PREGNANT AND NON-PREGNANT WOMEN. THE ENZYMATIC ACTIVITY OF THE SERA WAS DETERMINED AT pH 7.4 AND IS GIVEN IN MG $\beta$-NAPHTHYLAMINE/100 ML SERUM/HOUR (TUPPY AND NESVADBA, 1957)

| Sera | | Substrate $\beta$-Naphthylamide of: | | | | | |
|---|---|---|---|---|---|---|---|
| | | Glycine | DL-Alanine | L-Leucine | L-Cystine | L-Cysteine | DL-Methionine |
| 37 to 40th week pregnant women | S 43 | 16.25 | 79.2 | 47.2 | 6.05 | 0.60 | 2.75 |
| | S 44 | 19.03 | 81.2 | 49.6 | 5.80 | 0.70 | 3.20 |
| | S 45 | 16.40 | 77.6 | 50.4 | 5.05 | 0.58 | 2.95 |
| Mean ($M_1$) | | 17.23 | 79.3 | 49.1 | 5.63 | 0.63 | 2.97 |
| Non-pregnant women | S 71 | 13.7 | 44.8 | 15.2 | 0.50 | 0.58 | 3.6 |
| | S 72 | 7.35 | 26.1 | 12.8 | 0.40 | 0.56 | 2.5 |
| | S 73 | 5.85 | 25.6 | 13.6 | 0.40 | 0.40 | 2.25 |
| Mean ($M_2$) | | 8.97 | 32.2 | 13.9 | 0.43 | 0.51 | 2.8 |
| $M_1/M_2$ | | 1.9 | 2.5 | 3.5 | 13.1 | 1.2 | 1.1 |

out effect on oxytocinase, though, both systems are inhibited by chelating agents, e g ethylenediamine tetraacetic acid. Further research on these lines may well lead to an early clarification of these problems and so to a better understanding of the fate of oxytocin in the organism.

An inactivation of oxytocin different from that discovered by TUPPY and NESVADBA (1957) has been described by RESSLER (1958). It involves cleavage of the intramolecular disulphide bond of oxytocin and the formation of intermolecular disulphide bonds between two or more molecules of the hormone. A less soluble dimer or other higher molecular weight form is obtained This type of inactivation was found to occur under mildly alkaline conditions *in vitro*. It is not yet known whether oxytocin can undergo such changes *in vivo*.

# V

## OXYTOCIN AND LABOUR

THE TERM "oxytocic" is derived from the Greek ὀξύς (swift) and τόκος (birth). Oxytocin is therefore aptly named, for it accelerates labour by eliciting or reinforcing contractions of the uterine smooth muscle in many species including man This property finds clinical application for induction of labour and correction of uterine inertia. Oxytocin has a peripheral site of action—it acts directly on receptors in the smooth muscle cells of the uterus—so that *in vitro* preparations can be used to demonstrate its oxytocic or uterotonic action (Fig 24) In addition to eliciting uterine contractions, oxytocin appears to enhance the sensitivity of the myometrium to other stimuli (CSAPO, 1954)

From electrophysiological studies on the rat uterus (JUNG, 1957), the primary action of oxytocin on uterine muscle seems to be to lower the membrane potential This is followed by a series of tetanic action potentials which, with low doses (e g., 0 001 I U i v), are attended by a mechanical contraction. The latent period before these discharges appear depends upon the hormone concentra tion With increasing oxytocin doses, the amplitude of the action potentials as well as the time that elapses before the peak potential is reached, both decrease But the most remarkable phenomenon is that the muscle remains contracted long after the action potentials have died away (Fig 25) It may be concluded from this that the pro-

out effect on oxytocinase; though, both systems are inhibited by chelating agents, e.g. ethylenediamine tetraacetic acid. Further research on these lines may well lead to an early clarification of these problems and so to a better understanding of the fate of oxytocin in the organism.

An inactivation of oxytocin different from that discovered by TUPPY and NESVADBA (1957) has been described by RESSLER (1958) It involves cleavage of the intramolecular disulphide bond of oxytocin and the formation of intermolecular disulphide bonds between two or more molecules of the hormone. A less soluble dimer or other higher molecular weight form is obtained This type of inactivation was found to occur under mildly alkaline conditions *in vitro*. It is not yet known whether oxytocin can undergo such changes *in vivo*

oxytocin levels that obtain in the sequence of events in childbirth. This question, moreover, is of considerable biological interest.

There is really only one finding at odds with the supposition that oxytocin may be implicated in labour,

[ 0 3 mV

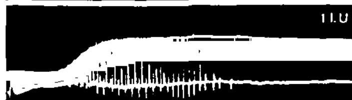
a




b



c




d



Fig 25 The action potentials and mechanical responses of a rat uterus under the influence of increasing doses of oxytocin Following large doses the tonus stays high even when the action potentials have subsided In trace "d" the action potentials are very low and are of a different kind as can be seen even at this recording speed (Jung, 1957)

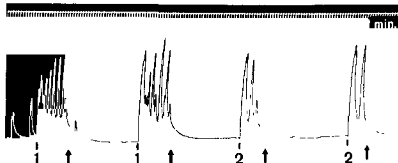



Fig 21 The effect of synthetic oxytocin (Syntocinon) on an isolated strip of human uterine muscle Caesarian section was performed on the 15th day after term At 1, 0 02 I U. and at 2, 0 01 I U of Syntocinon was added to the organ bath (50 ml) At ↑ the bath was washed out twice Time 1 minute The response was dependent upon the dose

tracted shortening of the uterine smooth muscle, the so-called "tetanus uteri," elicited by high doses of oxytocin, is not really tetanus in the true sense of the word, but a contracture However, recent studies (Evans *et al.*, 1958) suggest that the action of oxytocin on uterine muscle is not exclusively mediated via changes in membrane potential: rat uteri were immersed in Ringer's solution in which sodium was replaced by potassium. Although the uteri had no measurable membrane potential they still contracted in response to a number of smooth muscle stimulants including oxytocin It seems possible, therefore, that oxytocin may activate the contractile elements without invoking the mechanism of membrane depolarisation.

A question of much interest to the clinician, and especially to the obstetrician, is whether oxytocin is actually a physiological factor in the process of labour If this were the case, it would be interesting to know the physiological

sary to administer roughly eight times more oxytocin in the 20th week and twice as much in the 30th week than at term (POSE and CALDEYRO-BARCIA, 1958). The increase in sensitivity is mainly confined to the corpus (Fig. 26)

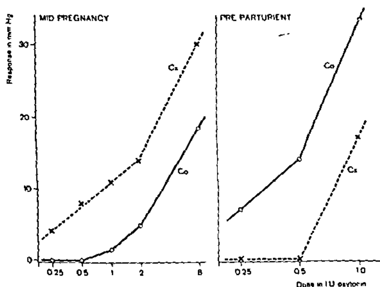



Fig. 26. Increase in the sensitivity to oxytocin of the corpus uteri at the end of pregnancy. Simultaneously recorded responses of corpus (Co) and cervix (Cx) are plotted against the dose of oxytocin (i.v.) at two stages of pregnancy. At mid pregnancy (22 weeks) the cervical responses are consistently greater than those of the corpus, whilst in a cow 36 hours before delivery the corpus responses are the greater (FITZPATRICK, 1957)

(FITZPATRICK, 1957) which, by its contractions, contributes to the expulsion of the foetus during labour. The sensitivity of the cervix does not appear to be enhanced at term. This is in keeping with the passive rôle of this part of the uterus in the process of labour. So pronounced is the increase in uterine sensitivity to oxytocin at term, that it

namely oxytocin is not found in higher concentrations in the blood of women in labour (HAWKER and ROBERTSON, 1956). This clearly cannot be ignored, but it should be pointed out that the methods in current use for estimating oxytocin in the blood are beset with considerable difficulties (see Chapter IV). The results of such estimations are frequently contradictory and a certain caution should be exercised in evaluating them, bearing in mind that transient, periodic fluctuations in the blood oxytocin level could conceivably occur and pass unnoticed Experimental evidence to be discussed below suggests that the blood oxytocin level may well be subject to periodic fluctuations of this kind.

There was formerly a great deal of discussion as to whether or not labour was retarded by hypophysectomy or sectioning of the pituitary stalk, since the experimental findings (recently reviewed by HARRIS, 1955) were contradictory This subject has lost some of its interest, for it is almost certain that the so-called posterior pituitary hormones are produced in the hypothalamus, and the *median eminence of the tuber cinereum is known to be* as much a part of the neurohypophysis as is the posterior lobe itself

The view that oxytocin plays a part in labour finds support in the complete analogy between normal, spontaneous uterine contractions and uterine motility elicited by oxytocin appropriately administered in correct dosage This applies to the shape, intensity, frequency, duration, tonus as well as to the co-ordination of the contractions in women (CALDEYRO-BARCIA and POSEIRO, *1958*)

Evidence that oxytocin participates in the physiological processes of normal birth is likewise furnished by the observation that uterine sensitivity to this hormone *increases at term* For a comparable response it is neces-

an be employed to predict with a high degree of probbility whether rupture of the membranes will induce abour (SMYTH, 1958a, 1958c): if a uterine contraction elicited by intravenous administration of 0.01 I U./min xytocin up to a total of 0.03 I.U. the chances of successal surgical induction are good (Fig. 27); if the uterus reponds only to greater amounts of oxytocin, the immediate hances of successful surgical induction are slight. Table 'II shows that the oxytocin sensitivity of the uterus is also useful guide to the probable date of spontaneous onset f labour (NIXON and SMYTH, 1958) The response of the terus to oxytocin is, further, a useful indication as to hether medical induction of labour is likely to be sucessful (MÜLLER, 1958).

TABLE VII

HE SCALE OF SENSITIVITIES APPLICABLE FOR ASSESSING THE PROBABLE ONSET OF LABOUR OR FALSE LABOUR (NIXON AND SMYTH, 1958)

| Sensitivity—total units oxytocin | Probable days to onset |
|---|---|
| 0 01 | same day |
| 0 02 | same day or next day |
| 0 03 | next day |
| 0 04 | 2 days |
| 0 06 | 3 to 4 days |
| 0 08 | 5 to 6 days |
| 0 10 | a week |

An observation by GUNTHER (1948) has a close bearng on the question of oxytocin secretion during labour he noted in a lactating woman in labour that each uterine ontraction was accompanied by a spontaneous ejection of milk The phenomenon of milk ejection is now known

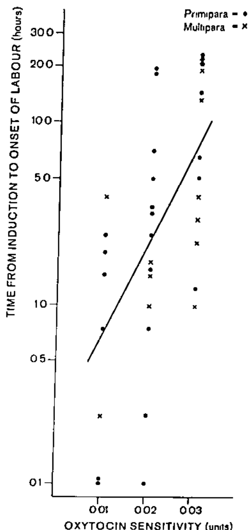



Fig 27 Relation between oxytocin sensitivity and onset of labour after amniotomy in patients sensitive to less than 0·01 I U oxytocin The correlation is significant at 200 : 1 probability (SMYTH, 1958a)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Lactating cats (4) | 2 750 | 2,269 | 2,440<br>±722.9 | 1,020<br>±422.1 | 90<br>±30.1 | 37<br>±14.3 | 108<br>±32.3 | 45<br>±18.4 | 2.38<br>— | 3<br>— |
| Normal dogs (8) | 12,313 | 5,248 | 7,050<br>±420 | 6,950<br>±505 | 64<br>± 1.6 | 629<br>± 8.4 | 142<br>± 9.3 | 138.0<br>±14.7 | 1.03<br>— | —<br>— |
| Female dogs, Delivery (3) | 14 500 | 5 941 | 6,600<br>±1,830 | 3 500<br>±1,021 | 48.0<br>— | 28.0<br>— | 113<br>— | 65<br>— | 1.93<br>— | 4<br>— |
| Female dogs, Lactating (4) | 12,000 | 5 150 | 6,600<br>±1,480 | 1,220<br>± 203 | 61<br>±13.9 | 13<br>± 4.6 | 131<br>±24.4 | 23<br>± 4.9 | 5.41<br>— | 6<br>— |

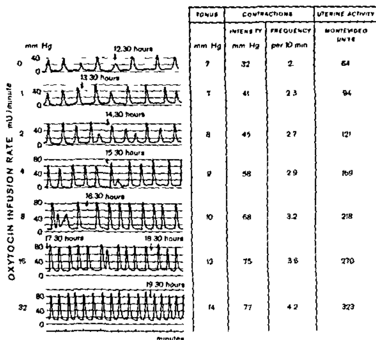
Data for dogs are taken from a paper by DICKER and TYLER (1953a) Number of animals in parentheses

TABLE VIII

RELATION BETWEEN VASOPRESSOR (V) AND OXYTOCIC (O) ACTIVITIES OF THE PITUITARY GLAND AND BODY WEIGHT AND BODY SURFACE, AND THE EFFECT OF DELIVERY AND LACTATION, IN ADULT RATS, GUINEA-PIGS, CATS AND DOGS (DICKER AND TYLER, 1953b)

| | Body wt (g) | Surface (cm²) | mU/gland | | mU/100g body wt | | mU/100 cm² | | | Average no. of |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | V | O | V | O | V | O | V/O | littermates |
| Adult rats (18) | 230 | 325 | 350 ± 40.0 | 320 ± 45.0 | 150 ± 9.9 | 130 ± 9.6 | 100 ± 8.1 | 95 ± 9.3 | 1.14 | — |
| Female rats, Delivery (5) | 186 | 288 | 330 ± 51.4 | 190 ± 24.9 | 181 ± 29.5 | 105 ± 15.0 | 116 ± 18.3 | 67 ± 8.9 | 1.73 | 9 |
| Female rats, Lactating (6) | 216 | 314 | 240 ± 56.6 | 130 ± 23.8 | 134 ± 50.5 | 71 ± 21.8 | 85 ± 27.5 | 45 ± 11.8 | 1.85 | 10 |
| Adult guinea pigs (16) | 411 | 461 | 600 ± 122.0 | 240 ± 70.4 | 145 ± 80.8 | 61 ± 12.7 | 128 ± 23.7 | 54 ± 15.9 | 2.43 | — |
| Female guinea-pigs, Delivery (4) | 538 | 544 | 770 ± 134.2 | 460 ± 187.2 | 135 ± 28.3 | 79 ± 20.6 | 135 ± 29.4 | 81 ± 22.2 | 1.88 | 3 |
| Female guinea-pigs, Lactating (2) | 682 | 620 | 470 | 129 | 69 | 19 | 76 | 21 | 3.64 | 3 |
| Normal cats (4) | 3,140 | 2,302 | 3,870 ± 262.0 | 3,250 ± 460.3 | 126 ± 10.3 | 108 ± 10.9 | 168 ± 9.6 | 141 ± 18.3 | 1.19 | — |

tinuously recorded and calculated in Montevideo units (amplitude of contractions in mm. Hg x frequency of contractions per ten minute interval). There was found to be a clearly defined relationship between the amount of oxytocin infused and the resultant uterine activity (Fig. 28) Indeed, if uterine activity and oxytocin dose are plotted on a suitable scale, a rectilinear relationship is obtained




| TONUS | CONTRACTIONS | | UTERINE ACTIVITY |
|---|---|---|---|
| | INTENSITY | FREQUENCY | MONTEVIDEO UNITS |
| mm Hg | mm Hg | per 10 min | |
| 7 | 32 | 2. | 64 |
| 7 | 41 | 2.3 | 94 |
| 8 | 45 | 2.7 | 121 |
| 9 | 58 | 2.9 | 169 |
| 10 | 68 | 3.2 | 218 |
| 13 | 75 | 3.6 | 270 |
| 14 | 77 | 4.2 | 323 |

Fig 28 Seven sections from a record of uterine contractility obtained in a full term pregnant woman Section O shows the spontaneous contractility The subsequent sections show the contractility produced by the infusion of oxytocin at the rates of 1, 2, 4, 8, 16 and 32 mU/min The average values of the tonus, intensity, frequency and uterine activity are indicated for each section In section 16 the recording has been temporarily interrupted (CALDEYRO-BARCIA *et al*, 1957b)

to be due to the release of oxytocin from the neurohypophysis (see Chapter VI). This observation would therefore imply that there is a periodic rise in oxytocin secretion during labour which coincides with the uterine contractions. A similar observation has recently been made in rabbits (CROSS, 1958b): it has been estimated from the milk-ejection pressure that some twenty mU. of oxytocin may be released during expulsion of a foetus.

Another important finding in this connection is that electrical stimulation of the supraoptico-hypophyseal tract in anaesthetised pregnant rabbits at term can induce labour in the same way as intravenous administration of 50 to 200 mU. of oxytocin (CROSS, 1958b)

In conclusion it should be mentioned that the oxytocin content of the hypophysis decreases during labour (Table VIII) in both rats (DICKER and TYLER, 1953b, ACHER and FROMAGEOT, 1957) and dogs (DICKER and TYLER, 1953a) There is a certain parallel between this finding and the observation that the oxytocic activity of the urine of pregnant women is higher than that of the urine of men and non-pregnant women (COCKRILL *et al.*, 1934).

There is thus quite a considerable body of evidence to support the view that oxytocin may well be one of the physiological regulators of uterine activity during labour

If oxytocin actually were a physiological factor in the process of birth it would be of considerable importance for the obstetrician to know the amount of oxytocin secreted during labour For this reason the direct tocometric investigations of CALDEYRO-BARCIA *et al* (1957a, 1957b, 1958) in women shortly before delivery are highly interesting These investigators administered synthetic oxytocin (Syntocinon) by intravenous drip infusion in various doses, each dose for one hour Uterine activity was con-

motility at the end of the first stage and in the second stage can be simulated by giving about 8 mU./min.; and the most powerful contractions occurring during the second stage can be imitated by administering about 16 mU./min.* This latter infusion rate may occasionally elicit uterine contractions more powerful than those occurring in spontaneous labour. From these findings CALDEYRO-BARCIA and his co-workers conclude that the physiological secretory activity of the neurohypophysis during the different stages of labour is probably within this range.

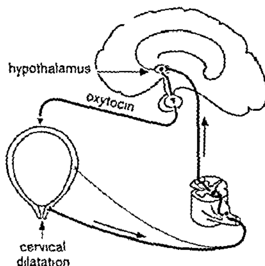



Fig 30 Diagram of the FERGUSON reflex Dilatation of the cervix, and less consistently dilatation of the corpus augments uterine motility by reflex release of oxytocin (CALDEYRO-BARCIA *et al*, 1957a)

*These are average values pertaining to physiological conditions In pathological states the irritability of the uterus may be different Thus in toxaemia of pregnancy sensitivity to oxytocin is enhanced, while in polyhydramnios and myxoedema, and in elderly primigravidae, it is lowered (CALDEYRO BARCIA and POSEIRO, 1958)

(Fig. 29). Uterine activity at the commencement of labour (cervix 2 cm.) can be simulated by giving an intravenous drip infusion of 1 to 2 mU./min. of oxytocin; uterine

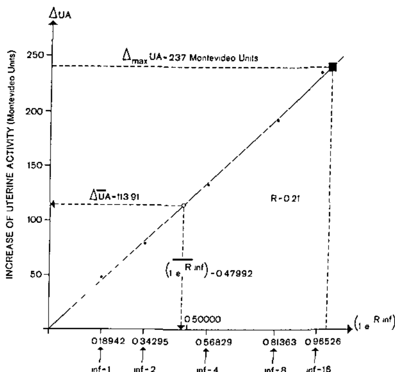



Fig. 29 The exponential relationship between the oxytocin infusion rate and the increase of uterine activity in full term pregnant women The increase of uterine activity ($\Delta$UA) is plotted against ($1 e^{R\,inf}$) The values of infusion rate (inf) are indicated below the corresponding value on the abscissa The straight line represents the theoretical function Black circles show the experimental average values of the 8 records The white circle is the baricentre of the straight line corresponding to the mean value of both coordinates The black square corresponds to abscissa equal to one and ordinate equal to ($\Delta_{max}$UA) (CALDEYRO BARCIA *et al.*, 1957b)

this reflex also operates during labour.* It is conceivable that a sort of chain reaction could occur as labour progresses: dilatation of the cervix leading to release of oxytocin, increased secretion of oxytocin evoking contraction of the corpus and fundus, and this in turn causing further dilatation of the cervix, and so on.

It is instructive to compare the levels of "physiological secretion" of oxytocin in the human female, as estimated by CALDEYRO-BARCIA, with the oxytocin doses recommended by obstetricians for induction of labour or correction of uterine inertia. THEOBALD, the originator of oxytocin therapy by intravenous drip infusion (1948), recently recommended an initial infusion rate of 0.7 to 1.8 mU./min for induction (1957), i.e. a dosage almost identical with CALDEYRO-BARCIA's "physiological" level at the commencement of the first stage in spontaneous labour. The last few years, in fact, have witnessed quite a general trend in the literature (see Table IX) towards oxytocin infusion rates of the order of the "physiological" levels indicated above. There would thus appear to be a valid case for regarding well-conducted intravenous infusion of oxytocin as physiological substitution therapy rather than pharmacological intervention.

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* *The* FERGUSON *reflex cannot be demonstrated in non-pregnant animals* (CROSS, 1958a)

The rise in oxytocin secretion as labour proceeds is explained by the FERGUSON reflex (1941). Dilatation of the uterus, but more especially of the cervix, acts as a secretory stimulus on the neurohypophysis (Fig 30) FERGUSON made his observations on postpartal animals, but it has recently been demonstrated (CROSS, 1958b) that

TABLE IX

DRIP INFUSION RATES FOR THE INTRAVENOUS ADMINISTRATION OF OXYTOCIN IN WOMEN (CALDEYRO-BARCIA *et al*, 1957a)

| Infusion rate mU /min | Author |
|---|---|
| 0 5 – 2 | Greenhill (1955) |
| 1 25 – 2 5 | Nixon and Smyth (1957) |
| 2 – 4 | Carey (1954) *Faris and Kahlenberg (1954)* |
| 2 5 – 5 – 10 | Theobald (1948) Bishop (1955) White (1955) |
| 4 – 8 | Holland (1955) Ratzan and Shulman (1955) |
| 9 | Labate and Barbaro (1951) |
| 4 – 15 | Lizana Farías and Fernández Popelaire (1953) |
| 4 – 25 | Bosch and Kaser (1956) |
| 5 – 20 | Bainbridge, Nixon, Schild and Smyth (1956) |
| 6 – 16 | Engstrom and Ohlson (1956) Varangot and Côte (1955) Richon and *Braye (1955)* |
| 7 – 10 | Parker and Roberts (1954) Cazzola (1955) Mathew (1956) |
| 7 – 15 | Savi (1956) Fuster, Queralt and Varela (1954) Williams and McMahon (1956) |
| 7 – 21 | Moore and D'Esopo (1955) |
| 7 – 25 | MacKenzie (1954) |
| 10 – 20 | Hellman (1949) Hellman, Harris and Reynolds (1950) Tisné (1955) |
| 15 – 25 | Pigeaud (1952) Cantarow (1954) |
| 30 | Hukill (1955) |
| 10 – 40 | Piton (1952) Kaufman (1953) Dexeus and Segur (1955) |
| 30 – 40 | Creze and *Rouchy (1957)* |
| 30 – 60 | Molina Yañez (1955) |

ferous ducts and sinuses, can be drawn out by the mechanical action of suckling or milking. The greater part of the milk, however, is in the alveoli and finer ducts which cannot be evacuated by suckling or milking alone, but only with the aid of the contractile system of the mammary gland. The milk must be forced into the large ducts converging on the nipple. This process is known as milk ejection or let down.

The contractile system responsible for the milk ejection reaction consists of special myoepithelial cells (basket cells) of ectodermal origin, not, as might be imagined, of smooth muscle cells (RICHARDSON, 1949). Basket cells can be demonstrated throughout the whole mammary gland, they are present in large numbers, intimately associated with the alveoli and fine ducts, and are therefore able to exercise the necessary contractile force. The milk-ejection reaction which is accompanied by a measurable rise in pressure in the mammary gland, results from the direct action of oxytocin on the myoepithelial cells (LINZELL, 1955).

The rise in pressure in the ducts converging on the nipple in response to oxytocin is so characteristic that in suitable tests it can be employed to detect small amounts of oxytocin (CROSS and HARRIS, 1951/52, VAN DYKE *et al.*, 1955, BERDE and CERLETTI, 1957), and for the bioassay of oxytocin preparations. Figure 31 shows part of the tracing from an experiment in which a synthetic oxytocin preparation (Syntocinon) was compared with International Standard Pituitary (Posterior Lobe) Powder on the mammary gland of a lactating doe rabbit, using SCHILD's (1942) four-point assay design. It should, however, be added that the milk let-down reaction is not entirely specific for oxytocin: the myoepithelial cells show a greater or lesser response with other allied polypeptides

# VI

## OXYTOCIN AND LACTATION

As far as clinical medicine is concerned oxytocin is still primarily an oxytocic However, its action on the uterus is by no means its only important biological property Oxytocin is also mainly responsible for the effect of the neurohypophysis on the lactating mammary gland This effect is just as specific as the action on the uterus, and, seen from a biological viewpoint, it is just as important There is a coherent body of convincing data to indicate that a reflex liberation of oxytocin occurs whenever a mother takes her child to the breast, whenever a young animal suckles or a cow is milked A neurohormonal reflex involving oxytocin would seem to be essential for complete emptying of the mammary gland So specific is this action of oxytocin that a response can be detected in the mammary gland of a lactating woman with 0 01 I U in a single intravenous injection (Beller *et al*, 1958) and with as little as 0 1 to 0 2 mU /min i v (Theobald, 1958) Similarly in the lactating rabbit 0 0005 I U of oxytocin i v (Van Dyke *et al*, 1955, Berde and Cerletti, 1957) may elicit a measurable response

The rôle of oxytocin in lactation has been thoroughly investigated in recent years (see for example Petersen, 1944, Cross and Harris, 1951/52, Cross, 1955a, Folley, 1956, Cowie and Folley, 1957, Labouche, 1957) We now know that only a part of the milk present in the mammary gland, namely the milk contained in the large lacti-

which can be regarded as the reflex centre, and results in a release of oxytocin by the neurohypophysis The hormone is carried to the mammary gland in the blood stream and constitutes the efferent (humoral) arc of the neurohormonal reflex.

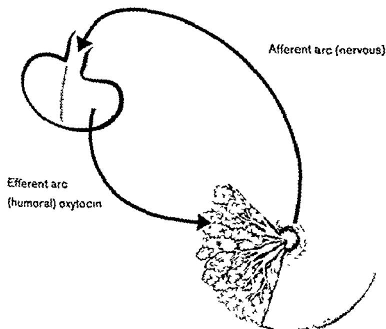



Fig 32 Schematic representation of the milk-ejection reflex Mechanical stimulation of the mammilla activates the afferent (nervous) reflex arc Oxytocin is then released from the neurohypophysis to form the efferent arc of the neurohormonal reflex

The presence of a humoral factor in the blood, involved in milk ejection, has been demonstrated to particularly good effect in experiments on the isolated cow udder (PEETERS *et al*, 1947) Blood taken from a cow prior to application of the milking stimulus and perfused

Vasopressin, for example, has a weak, but measurable effect (Fig. 4) (VAN DYKE *et al.*, 1955), and all the oxytocin analogues we have so far investigated in the milk-ejection test have been found to produce a greater or lesser response (BERDE *et al.*, 1957) (Table II).

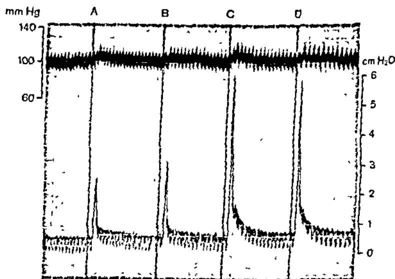



Fig 31 The action of oxytocin on the pressure in a duct of the mammary gland of a lactating rabbit under urethane anaesthesia Above blood pressure, below milk ejection pressure Time 30 sec The i v injections were made at intervals of 3 min A = 0 001 I U and C = 0 008 I U of International Standard Pituitary (Posterior Lobe) Powder, which contains natural oxytocin B = 0.000083 ml and D = 0 000166 ml of a Syntocinon (synthetic oxytocin) solution Taken from a four point assay.

The release of oxytocin during suckling or lactation is brought about by the milk-ejection reflex mechanical stimulation of the mamilla is the physiological stimulus activating the afferent (nervous) arc of the reflex (Fig 32) The stimulus is conducted to the hypothalamus,

which can be regarded as the reflex centre, and results in a release of oxytocin by the neurohypophysis. The hormone is carried to the mammary gland in the blood stream and constitutes the efferent (humoral) arc of the neurohormonal reflex.

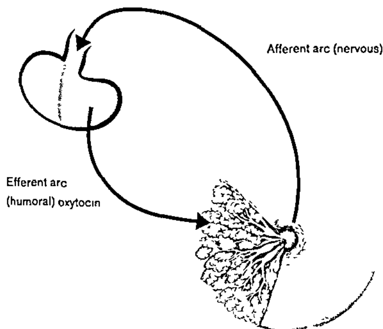



Fig 32 *Schematic representation of the milk ejection reflex* Mechanical stimulation of the mammilla activates the afferent (nervous) reflex arc Oxytocin is then released from the neurohypophysis to form the efferent arc of the neurohormonal reflex

The presence of a humoral factor in the blood, involved in milk ejection, has been demonstrated to particularly good effect in experiments on the isolated cow udder (PEETERS *et al*, 1947) Blood taken from a cow prior to application of the milking stimulus and perfused

through an isolated udder had a much weaker milk let-down effect than blood taken from the same cow following such a stimulus (Table X). Although measurements of the blood oxytocin level in women have not revealed any increase during lactation (HAWKER, 1958), there is evidence to suggest that a humoral factor similar to oxytocin is present in human female blood during suckling or after stimulation of the mamilla. Thus milk ejection occurs in both breasts even if only one nipple is stimulated (NEWTON and NEWTON, 1948) and stimulation of the nipple at the end of pregnancy, during labour or in the puerperium, results in heightened uterine motility (LORAND and ASBOT, 1952)

TABLE X

EXPULSION OF MILK FROM THE TWO HALVES OF A BOVINE UDDER, PERFUSED WITH BLOOD FROM THE SAME COW BEFORE AND AFTER APPLICATION OF THE MILKING STIMULUS (PEETERS *et al*, 1947)

| Experiment No | Amount of milk expelled from the udder | |
|---|---|---|
| | Left half perfused with blood taken before milking stimulus applied ml | Right half perfused with blood taken after milking stimulus applied ml |
| 1 | 160 | 360 |
| 2 | 270 | 520 |
| 3 | 215 | 345 |
| 4 | 390 | 500 |

The reflex nature of the milk-ejection reaction can be seen from the latent period of the response in the rabbit which amounts to 7 to 15 seconds following intravenous injection of oxytocin, 13 to 25 seconds following electrical

stimulation of the supraoptico-hypophyseal tract, and 30 to 90 seconds after commencement of suckling (Table XI) (HARRIS, 1955). The nervous component of the reflex appears to involve both adrenergic and cholinergic synapses (GROSVENOR and TURNER, 1957b). The reflex can be conditioned both in animals and the human female (WALLER, 1938) and is abolished by anaesthesia, though oxytocin elicits milk ejection even in anaesthetised animals (GAINES, 1915; CROSS, 1955b, COWIE and FOLLEY, 1957; GROSVENOR and TURNER, 1957a, 1958a). Emotional influences can adversely affect the milk-ejection reflex both in animals (ELY and PETERSON, 1941, CROSS, 1952/53a) and in the human female (NEWTON and NEWTON, 1948) Such inhibitory effects would appear to involve the sympathico-adrenal system (CROSS, 1952/53b, 1955b)

TABLE XI

TIME FACTORS IN THE MILK-EJECTION REFLEX IN THE RABBIT (HARRIS, 1955)

| | Milk-ejection process observed after | |
|---|---|---|
| | Stimulation of the supraoptico-hypophyseal tract | Normal suckling |
| Latent period | 13-25 sec | 30-90 sec |
| Duration of response | 2- 7 min | 2-5½ min |

In line with the increased liberation of oxytocin, the hormone content of the hypophysis is depleted during lactation This has been observed in the rat (DICKER and TYLER, 1953b, ACHER and FROMAGEOT, 1957), in the dog

(DICKER and TYLER, 1953a; VAN DYKE *et al.*, 1957), in the guinea pig and in the cat (DICKER and TYLER, 1953b) (Table VIII). However no decrease in hypophyseal hormone content has been observed in the lactating goat (FOLLEY, 1956).

In addition to promoting the withdrawal of milk (galactokinetic effect), oxytocin appears to participate indirectly in the actual secretion of milk (galactopoietic effect). Certain experimental findings (BENSON and FOLLEY, 1956a) indicate that oxytocin acts as a stimulus to the *secretion of prolactin* and/or other anterior-lobe hormones responsible for milk production. Involution of the mammary gland such as is observed when a lactating animal is deprived of its young can be retarded by oxytocin injections No such effect can be demonstrated in hypophysectomised animals (BENSON and FOLLEY, 1956b, 1957). This view finds support in the observation (DESCLIN, 1956a, 1956b) that a clear-cut luteotrophic activity can be demonstrated in non-pregnant rats several days after a single injection of oxytocin The luteotrophic hormone (LTH) is, however, identical with prolactin. Oxytocin is assumed to bring about the release of prolactin by acting directly on the adenohypophysis.*

Direct anatomical communication between the neurohypophysis and the adenohypophysis is actually provided in the portal circulation of the hypophysis (POPA and FIELDING, 1930, WISLOCKI, 1938; GREEN and HARRIS, 1946/48) small arteries from the internal carotid and the

*Apparently, this mechanism is not unique Another example of a hypothalamic hormone stimulating the anterior pituitary is the corticotrophin releasing factor or CRF This polypeptide, which chemically seems to be related to vasopressin, is found in the hypothalamus and acts on the adenohypophysis enhancing the secretion of ACTH (SAFFRAN, 1958, GUILLEMIN *et al*, 1957)

posterior communicating artery supply the pars tuberalis of the adenohypophysis Their capillaries extend into the median eminence (neurohypophysis). The blood from this primary plexus collects in portal trunks and then passes to a second capillary bed (the sinusoids) of the *pars distalis* which is a part of the adenohypophysis. The vascular system is thus a circuit containing two capillary beds, so that any neurohypophysial factors could act directly on the adenohypophysis (Fig. 33).

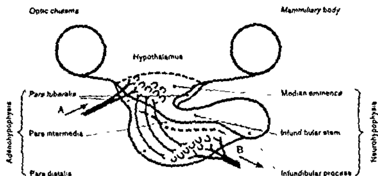



Fig 33 The portal circulation of the hypophysis in the rabbit blood in artery A first circulates through a part of the neurohypophysis The blood from the capillaries then collects in portal trunks and is distributed through a second capillary network, so that it also circulates through a part of the adenohypophysis before leaving the gland by vein B This diagram is based on two illustrations in a book by HARRIS (1955)

The view that oxytocin might exercise a prolactin-releasing effect has not gone uncontested (GROSVENOR and TURNER, 1958b), but the idea is none the less an attractive one. It would mean that the organism was very thrifty with its oxytocin, for the latter on being released into the blood stream in response to the suckling stimulus would have two different actions on the mammary gland. One

would be a direct effect: contraction of the myoepithelial cells and let down of stored milk. The other would be an indirect effect mediated via the anterior pituitary and operating to maintain milk secretion

While the uterotonic action of oxytocin is widely employed in obstetrics, the clinical application of its effect on the mammary gland has been rather neglected in spite of the fact that milk let down can be promptly elicited in lactating women If oxytocin is administered by the intravenous route the latent period is only 22 to 37 seconds (NICKERSON *et al*, 1954). The latter years have seen an increase in the number of clinical publications reporting successful therapeutic application of this property of oxytocin, especially in postpartal engorgement of the breast and in other cases in which the mother seems unable to nurse the baby adequately (e.g, NEWTON and NEWTON, 1951, HAEGER and JACOBSOHN, 1953, VARANGOT and YBERT, 1955, PARDI, 1956, MASSANO, 1956, DOUGLAS *et al*, 1957, COLACURCI, 1957, STEWART and SLEZAK, 1958, HOLLENBACH, 1958) Since oxytocin is effective in such cases when administered intranasally (NEWTON and EGLI, 1958, BAUMGARTEN and WATZEK, 1959, WENNER, 1959) this therapy will probably gain greater popularity.

# VII

## SOME OTHER ACTIONS OF OXYTOCIN

IT WAS MENTIONED in passing in Chapter III that copulation is one of the stimuli which lead to release of oxytocin. As soon as the cow catches sight of the bull, its uterine motility increases (VANDEMARK and HAYS, 1952), and when the vagina and uterus of the cow are stimulated the blood oxytocin concentration rises from 120-300 μU /ml to 420-850 μU /ml (FITZPATRICK, 1957) With the aid of the milk-ejection reaction, it can be demonstrated that oxytocin is also released in the human female during coitus (HARRIS and PICKLES, 1953), the amount of hormone liberated being greater if an orgasm is attained (CAMPBELL and PETERSEN, 1953) However animal studies have revealed that mere mechanical stimulation of the uterus, vagina or vulva, without actual presence of the male, will cause a similar reaction The findings from a number of such studies are summarised in Table XII

It is assumed that oxytocin released during coitus, i e the uterine motility elicited by the hormone, accelerates the ascent of the spermatozoa in the female reproductive tract (VANDEMARK and HAYS 1952, CROSS, 1955a) This assumption seems justified, since in many species the ascent of the spermatozoa is far more rapid than can be accounted for by the inherent motility of the spermatozoa

TABLE XII

OXYTOCIN-LIKE RESPONSES DUE TO COITUS OR MECHANICAL STIMULATION OF THE UTERUS, THE VAGINA OR THE VULVA (FITZPATRICK, 1957)

| Species | Stimulus | Response | Author |
|---|---|---|---|
| Mare | Mating | Milk ejection | Hammond, 1936 |
| Man | Mating | Milk ejection | Pickles, 1953 |
| Man | Mating | Milk ejection | Harris & Pickles, 1953 |
| Man | Mating | Milk ejection | Campbell & Petersen, 1953 |
| Cow | Mating | Milk ejection | Hays & VanDemark, 1953 |
| Cow | Mating | Milk ejection | Campbell & Petersen, 1953 |
| Cow | Mating | Uterine motility | VanDemark & Hays, 1952, 1953 |
| Bitch | Mating | Uterine motility | Evans, 1933 |
| Goat | Manual cervix | Milk ejection | Andersson, 1951 |
| Cow | Manual cervix | Milk ejection | Hays & VanDemark, 1951 |
| Cow | Manual uterus | Milk ejection | Tgetgel, 1926 |
| Cow | Manual uterus | Milk ejection | Piana & Curto, 1950 |
| Cow | Manual uterus | Milk ejection | Usuelli *et al.*, 1952 |
| Cow | Manual uterus/cervix | Milk ejection | Hays & VanDemark, 1953 |
| Cow | Manual vulva/cervix | Uterine motility | VanDemark & Hays, 1951, 1954 |
| Rabbit | Stretching cervix | Uterine motility | Ferguson, 1941 |
| Rabbit | Manual vulva | Uterine motility | Krehbiel & Carstens, 1939 |
| Rabbit | Increased intrauterine pressure | Uterine motility | Reynolds, 1930 |
| Cat | Increased intrauterine pressure | Uterine motility | Schubel & Gehlen, 1933 |

themselves.* Moreover, immobile spermatozoa and sperm-free liquids are also transported along the reproductive tract. For example, transport of methylene blue, janus green B and iodochlorol has been observed in oestrous rabbits after artificial stimulation of the vulva (KREHBIEL and CARSTENS, 1939): the dyes reached the tubal end of the uterine horns two to five minutes after commencement of stimulation.

At the present time it is not known whether oxytocin plays a physiological rôle in menstruation, and for this reason it is difficult to interpret the therapeutic effect of the hormone in dysmenorrhoea (WOODBURY, 1950). It has been reported that oxytocin will abolish the signs and symptoms of dysmenorrhoea provoked by vasopressin As yet it is impossible to say whether this is an effect based on some physiological interplay between the two hormones, or whether the action of the oxytocin is purely pharmacological

On the whole we have good grounds for assuming that oxytocin participates in a number of reproductive functions in the female oxytocin released during coitus accelerates the ascent of spermatozoa in the reproductive system; oxytocin secretion resulting from dilatation of the cervix and body of the uterus increases the contractile activity of that organ during labour, and the release of oxytocin in response to stimulation of the mamilla facilitates the emptying of the mammary gland during lactation, simultaneously promoting the postpartum involution of the uterus Oxytocin may even be involved in menstruation. But what is the biological rôle of this hormone in the male? What functions does it perform in the female other than those mentioned above? Oxytocin has been

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*This question has recently been reviewed by FITZPATRICK (1957)

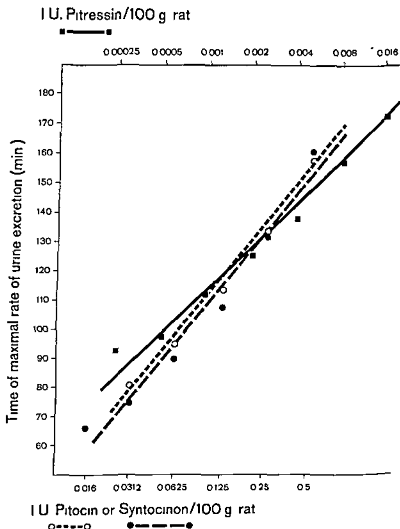



Fig 34 Comparison of the antidiuretic activity of vasopressin (Pitressin), natural oxytocin (Pitocin) and synthetic oxytocin (Syntocinon) in unanaesthetised rats The antidiuretic activity of oxytocin is about 1% of its oxytocic activity (BERDE and CERLETTI, 1956)

found in the blood of men and non-parturient and non-lactating women (BISSET *et al.*, 1956; BISSET and LEE, 1957). Moreover the pituitary glands of male animals contain considerable amounts of oxytocin (DICKER and TYLER, 1953b).

The first thing that springs to mind in seeking an answer to these questions is the influence of oxytocin on renal function. It has been demonstrated (VAN DYKE *et al.*, 1955, KONZETT, *et al.*, 1956, BERDE and CERLETTI, 1956) that oxytocin has an antidiuretic effect in high doses (Fig. 34). This is not surprising as oxytocin and vasopressin are rather similar in structure (see Chapter II) The antidiuretic activity of oxytocin is actually so slight, amounting to barely one per cent of its oxytocic activity, that it should perhaps be regarded as a pharmacological rather than a physiological property

Another action of oxytocin, and one of some biological significance, is that in the rat it causes increased excretion of water, sodium, potassium and chloride (FRASER, 1937, 1942, KUSCHINSKY and BUNDSCHUH, 1939, SCHAUMANN, 1949, BERDE and CERLETTI, 1956, CRONATTO *et al*, 1956, BRUNNER *et al*, 1956, 1957) The stimulant effect of oxytocin on diuresis was first described by FRASER (1937, 1942) who employed BURN's method (1931) in water-loaded rats These experiments have since been repeated (BERDE and CERLETTI, 1956) with synthetic oxytocin (Syntocinon) The results obtained bore out FRASER's observations, proving that the diuretic effect is due to oxytocin itself and not to some other factor that might be present in posterior-lobe extracts. The diuretic effect is brought out more clearly in thirsting rats (BRUNNER *et al*, 1956) and in rats loaded with physiological saline Under these conditions a clear dose-effect relationship can be obtained (Fig. 35)

Oxytocin has no diuretic effect in hypophysectomised animals. However, if such animals are given NaCl and desoxycorticosterone, they will give a diuretic response to oxytocin (CROXATTO and ZAMORANO, 1957). In a certain dose range oxytocin will antagonise the antidiuretic effect of vasopressin (FRASER, 1937; BRUNNER *et al.*, 1956

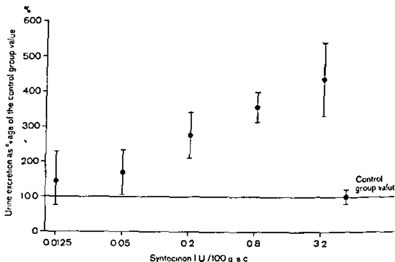



Fig 35 The diuretic action of oxytocin Male rats received 2 5 ml /100 g of 0 9% NaCl solution at body temperature orally, and were then given the test quantity of synthetic oxytocin (Syntocinon) s c Each oxytocin dose was administered to a group of 20 animals The urine excretion was measured for 5 hours, and the urine output of the control group was taken as 100% The mean values (•) and standard errors are shown

CROXATTO and ZAMORANO, 1957) This is a particularly interesting observation, since the two hormones are always released together, though in different proportions depending upon the nature of the stimulus (see Chapter III)

It should be added that the diuretic effect of oxytocin has not so far been demonstrated in man (CHALMERS *et al*,

solely to the rat. Thus if oxytocin is administered to a dog during a period of low urine flow (Fig. 36), it causes an increase mainly of sodium but also of potassium excretion (BROOKS and PICKFORD, 1957). No diuretic effect can be detected in water loaded dogs (ANSLOW and WESSON, 1955; VAN DYKE *et al.*, 1955; BROOKS and PICKFORD, 1957).

TABLE XIII

THE ACTION OF NATURAL OXYTOCIN (ORASTHIN) AND SYNTHETIC OXYTOCIN (SYNTOCINON) ON DIURESIS AND ENDOGENOUS CREATININE CLEARANCE IN THIRSTING RATS (BRUNNER *et al.*, 1957)

| Treatment | Dose mU/rat s c | Endog creatinine clearance ml/rat/min | Diuresis % | No of animals per group |
|---|---|---|---|---|
| Controls | | 1 22 ± 0 09 | 100 ± 10 6 | 12 |
| Orasthin | 62 5 | 1 25 ± 0 06 | 253 7 ± 23 4 | 13 |
| | 250 | 1 33 ± 0 13 | 260 2 ± 35 6 | 11 |
| Syntocinon | 62 5 | 1 29 ± 0 14 | 183 3 ± 22 6 | 13 |
| | 250 | 1 38 ± 0 12 | 241 5 ± 38 2 | 11 |

The mechanism of the diuretic effect of oxytocin is not yet fully understood In rats the creatinine clearance (Table XIII) was found to be unchanged following administration of oxytocin (BRUNNER *et al*, 1957) In conscious dogs, however, both the creatinine clearance, i e, the glomerular filtration rate (ALI, 1958), and the diodone clearance, i e the effective renal plasma flow (BROOKS and PICKFORD, 1957), were increased by oxytocin Both effects were antagonised by vasopressin (BROOKS and PICKFORD,

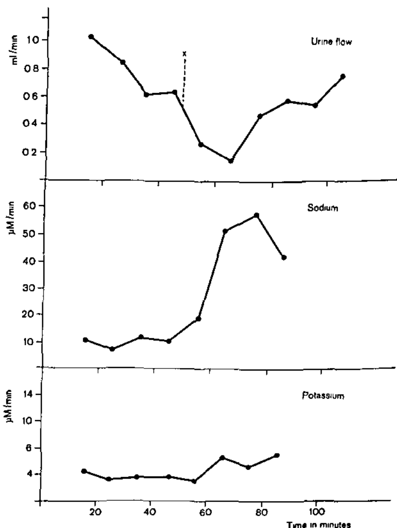



Fig 36 The effect of oxytocin (50 mU intravenously at x) on urine flow and Na and K excretion at a low rate of urine flow in a dog The increase of sodium excretion is especially marked (Brooks and Pickford, 1957)

1957, ALI, 1958). In the rat increased sodium and chloride excretion due to oxytocin is reinforced by mersalyl (Salyrgan) (SCHAUMANN and SCHMIDT, 1948), and oxytocin enhances excretion of water, sodium and potassium even when carbonic anhydrase in kidney tissue is fully inhibited by acetazolamide (CRONATTO and LABARCA, 1958).

It is still not altogether clear to what extent the diuretic effect of oxytocin is due to its action on the renal circulation or to what extent a direct influence of the hormone on the renal tubuli is responsible. Some insight into this problem was recently obtained by BROOKS and PICKFORD (1958) in experiments on dogs the action on renal blood flow seems to be independent of the action on electrolyte excretion The influence of oxytocin on renal clearances appears to be peripheral in origin, whereas its action on electrolyte excretion is mediated via an unknown central site. Certain effects of oxytocin only occur in the presence of vasopressin which suggests that the two hormones, acting together, play a part in the regulation of electrolyte excretion

Another open question is whether the effects of oxytocin on the circulation, which under certain conditions can be detected in animals and in man are of biological significance or merely pharmacological effects of high doses It has long been known that birds react to intravenously administered oxytocin with a steep fall in blood pressure (GADDUM, 1928) This effect is due to peripheral vasodilatation (PATON and WATSON, 1912) and is so characteristic that COON (1939) developed it as a method of bioassay It has since been adopted for this purpose in the *United States Pharmacopeia* (1955) and the *British Pharmacopoeia* (1953). Oxytocin analogues, e g valyl-oxytocin, have a similar depressor action on avian blood pressure.

In the human female, synthetic oxytocin (Syntocinon) administered by intravenous drip infusion during labour in amounts having a pronounced effect on the uterus, does not influence the blood pressure (Bösch and Käser, 1956a; Caldeyro-Barcia *et al.*, 1957a). However, rapid intravenous injection of high doses of oxytocin causes a transient and occasionally a rather steep fall in blood pressure (Schild *et al.*, 1951; Woodbury *et al.*, 1944, Mayes and Shearman, 1956, Caldeyro-Barcia *et al.*, 1957a) The drop in blood pressure may be accompanied by changes in the ECG, particularly when the dose exceeds 2 I U (Mayes and Shearman, 1956, Caldeyro-Barcia *et al*, 1957a) Animal experiments have not, however, revealed any involvement of the coronary circulation which could be held responsible for the ECG changes (Woodbury and Abreu, 1944).

The depressor effect of oxytocin in mammals shows considerable species differences (Woodbury and Abreu, 1944) and is not so readily explained as in birds Observations on chloralose-anaesthetised dogs (Berde and Cerletti, 1958b) suggest that the mechanism is probably a complex one: the fall in blood pressure that occurs in some of the animals with both synthetic and natural oxytocin, is not strictly dependent upon the dose. Thus 0 5 I U /kg intravenously may sometimes provoke just as great a fall in blood pressure (Fig 37) as a ten times higher dose Moreover, after a dose of 5 I U /kg., bradycardia is always observed, whereas 0 5 I U /kg usually results in tachycardia These alterations in heart rate are not secondary to the fall in blood pressure

The investigations of Woodbury and co-workers (1944) suggested that the effect of oxytocin on the blood pressure could not be due solely to vasodilatation and that a cardiac component was involved It was assumed that

oxytocin reduced the contractile force of the heart. It is thus particularly interesting that oxytocin has recently been reported to exert an opposite effect on the heart (LOCKETT, 1957): the pressor responses elicited by adrenaline, noradrenaline or splanchnic stimulation were potentiated by oxytocin in spinal cats. This is a cardiac action as in the heart lung preparation oxytocin lowered the threshold for the action of adrenaline and noradrenaline on heart size and force of contraction without influencing heart rate, oxygen uptake or coronary blood flow

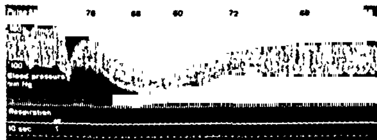



*Fig 37 The transient fall in blood pressure after 0.5 I U /kg Syntocinon i v. (at 1) that is sometimes observed in dogs anaesthetised by chloralose, under artificial respiration This effect is accompanied by slight tachycardia*

All these observations in animals and man merely show that high doses of oxytocin may affect cardiovascular function; they do not tell us whether this hormone has any influence on the circulation under physiological conditions. However, certain other findings hint at the possibility that oxytocin may in some way be involved in vascular phenomena. For example, in small amounts it causes vasodilatation in the pregnant canine uterus (AHLQUIST and WOODBURY, 1947) and can increase renal blood

flow in the dog by as much as 100 per cent (BROOKS and PICKFORD, 1957). But the most striking findings are those of DEMUNBRUN *et al.* (1954): glomerular filtration rate, renal plasma flow and tubular maxima are markedly reduced in dogs after neurohypophysectomy. In such animals renal haemodynamics can be restored to preoperative levels by the administration of oxytocin. Vasopressin has no such action. In view of this, these authors regard the regulation of kidney haemodynamics as one of the physiological functions of oxytocin This could explain why the hormone is found in both males and females



This short review makes no claim to completeness, but it will have served to show that much progress has been made in oxytocin research during latter years This progress whether in physiology, pharmacology or clinical medicine owes more than is sometimes realised to the chemist The isolation and synthesis of the hormone and the preparation of some of its analogues have brought about a "renaissance" in biological and clinical research into oxytocin Many problems have been elucidated, many more await an answer The most challenging of them all is the rôle of oxytocin in the male

# VIII

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